March 27, 2019

Centers for Disease Control and Prevention
CAPT Yon Yu, Pharm. D.
Senior Advisor for Regulatory Affairs
Division of Preparedness and Emerging Infections
National Center for Emerging and Zoonotic Infectious Diseases
1600 Clifton Rd; MS VI 8-4
Atlanta, Georgia 30329-4027

Re: K190302
Trade/Device Name: CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit, Influenza A Subtyping Kit (VER 2), Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2), Influenza A/H5 Subtyping Kit (VER 3)
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OZE, NSU, OEP, NXD, OQW, OOI
Dated: February 11, 2019
Received: February 12, 2019

Dear Dr. 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/CombinationProducts/GuidanceRegulatoryInformation/ucm597488.htm); 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 http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). 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 (http://www.fda.gov/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 R. Gitterman -S for

Uwe Scherf, Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K190302

Device Name
CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit, Influenza A Subtyping Kit (VER 2), Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2), and Influenza A/H5 Subtyping Kit (VER 3)

Indications for Use *(Describe)*
CDC Human Influenza Virus 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 Virus Real-Time RT-PCR Diagnostic Panel - Influenza A Subtyping Kit (VER 2):

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 Virus Real-Time RT-PCR Diagnostic Panel - Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2):

The Influenza B Lineage Genotyping 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 determination of the genetic lineage of human influenza B viruses as B/Victoria or B/Yamagata lineage 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]) 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 B lineage genotyping were established during a season when influenza B/ Victoria and B/Yamagata lineages were in circulation.

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.

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 Virus Real-Time RT-PCR Diagnostic Panel - Influenza A/H5 Subtyping Kit (VER 3):

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.

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 11, 2019

II. DEVICE INFORMATION

Proprietary Name:	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit, Influenza A Subtyping Kit (VER 2), Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2), and Influenza A/H5 Subtyping Kit (VER 3)
Common Name:	Influenza A/B Typing Kit, Influenza A Subtyping Kit, Influenza B Lineage Genotyping Kit, and 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 (K181736 and K172091)

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

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.

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.

The Influenza B Lineage Genotyping 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 determination of the genetic lineage of human influenza B viruses as B/Victoria or B/Yamagata lineage 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]) 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 B lineage genotyping were established during a season when influenza B/Victoria and B/Yamagata lineages were in circulation.

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.

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.

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. Additional options for real-time PCR instrumentation used with the CDC device to perform real-time RT-PCR assays are added to allow use of more recently available commercial instrument platforms.

VII. SUBSTANTIAL EQUIVALENCE COMPARISON

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K181736 and K172091), consisting of the Influenza A/B Typing Kit, Influenza A Subtyping Kit, Influenza B Lineage Genotyping Kit, and Influenza A/H5 Subtyping Kit will serve as the predicate for the proposed change. See tables 8-1 through 8-4 below for a detailed comparison to the predicate.

Table 8-1: Device Comparison

	Predicate Device	Proposed Device
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza A/B Typing Kit [K172091]**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza A/B Typing Kit**
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 Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument 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.	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.
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®, bioMerieux • 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	• 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
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™ 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® version 1.0.4.1 and Epsilon version 1.0.1 software

Table 8-2: Device Comparison

	Predicate Device	**Proposed Device**
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza A Subtyping Kit [K172091]**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza A Subtyping Kit**
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 Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument 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.	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.	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®, bioMerieux • 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	• 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
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™ 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® version 1.0.4.1 and Epsilon version 1.0.1 software

Table 8-3: Device Comparison

	Predicate Device	**Proposed Device**
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza B Lineage Genotyping Kit [K181736]**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza B Lineage Genotyping Kit**
Intended Use	The Influenza B Lineage Genotyping 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 Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument in conjunction with clinical and epidemiological information: • For the determination of the genetic lineage of human influenza B viruses as B/Victoria or B/Yamagata lineage 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]) 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 B lineage genotyping were established during a season when influenza B/Victoria and B/Yamagata lineages were found in approximately equal proportion. 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. 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.	The Influenza B Lineage Genotyping 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 determination of the genetic lineage of human influenza B viruses as B/Victoria or B/Yamagata lineage 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]) 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 B lineage genotyping were established during a season when influenza B/Victoria and B/Yamagata lineages were found in approximately equal proportion. 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. 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 B virus, lineages B/Victoria and B/Yamagata	Same
Specimen Types	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs 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	• 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®, bioMerieux • 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	• 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
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™ 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® verion 1.0.4.1 and Epsilon version 1.0.1 software

Table 8-4: Device Comparison

	Predicate Device	**Proposed Device**
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza A/H5 Subtyping Kit [K172091]**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel: Influenza A/H5 Subtyping Kit**
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 Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument 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.	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 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

	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.	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®, bioMerieux • 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	• 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
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™ 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® version 1.0.4.1 and Epsilon version 1.0.1 software

VIII. ANALYTICAL PERFORMANCE EVALUATION

Analytical Sensitivity - Limit of Detection (LOD) Equivalency Study

The LOD equivalency between the FDA-cleared CDC Human Influenza Real-Time RT-PCR Diagnostic Panel using the Applied Biosystems™ 7500 Fast Dx and either the Applied Biosystems™ QuantStudio™ Dx (QSDx) or the QIAGEN Rotor-Gene® Q MDx (QMDx) instrument was evaluated by testing 5-fold serial dilutions of characterized influenza viruses of known egg infectious dose 50% titer. Virus dilutions were prepared using a suspension of beta-propiolactone (BPL) treated A549 cells in viral transport medium (VTM) as diluent. Triplicate

samples of each dilution were extracted separately with the Roche MagNA Pure Compact RNA Isolation Kit. Each assay of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel was performed using Invitrogen SuperScript™ III Platinum® One-Step RT-PCR System (SuperScript) and Quanta qScript™ (qScript) enzyme systems. The acceptance criterion for LOD equivalence between the cleared Applied Biosystems™ 7500 Fast Dx and the investigational Applied Biosystems™ QSDx or the QIAGEN QMDx instrument was defined as a demonstration of 100% positivity (3 out of 3 replicates) at either the same endpoint concentration or within one 5-fold dilution. The results of the study are summarized by the virus tested in Tables 8-5 to 8-15. The lowest concentration at which each assay showed 100% positivity is highlighted. Each investigational instrument met the acceptance criterion when compared to the cleared instrument.

Table 8-5. 7500 Fast Dx vs. QSDx: Influenza A/Hong Kong/4801/2014 (H3N2)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx		QSDx		7500 Fast Dx		QSDx	
	InfA	H3	InfA	H3	InfA	H3	InfA	H3
$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.4}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	1/3 (+)	1/3 (+)	1/3 (+)	3/3 (+)
$10^{0.7}$	2/3 (+)	0/3 (+)	1/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	2/3 (+)
$10^{0.0}$	1/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-6. 7500 Fast Dx vs. QSDx: Influenza A/Michigan/45/2015 (H1N1)pdm09

Titer (EID_{50}/mL)[1]	SuperScript Enzyme						qScript Enzyme					
	7500 Fast Dx			QSDx			7500 Fast Dx			QSDx		
	InfA	pdm InfA	pdmH1	InfA	pdm InfA	pdmH1	InfA	pdm InfA	pdmH1	InfA	pdm InfA	pdmH1
$10^{3.6}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.2}$	3/3 (+)	3/3 (+)	0/3 (+)	3/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)	0/3 (+)	3/3 (+)	3/3 (+)	0/3 (+)	3/3 (+)
$10^{1.5}$	2/3 (+)	2/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	3/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-7. 7500 Fast Dx vs. QSDx: Influenza B/Montana/05/2012 (B/Victoria)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx		QSDx		7500 Fast Dx		QSDx	
	InfB	VIC	InfB	VIC	InfB	VIC	InfB	VIC
$10^{3.7}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{3.0}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.3}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	0/3 (+)	3/3 (+)	0/3 (+)
$10^{1.6}$	2/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)
$10^{0.9}$	3/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)
$10^{0.2}$	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-8. 7500 Fast Dx vs. QSDx: Influenza B/Massachusetts/02/2012 (B/Yamagata)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx		QSDx		7500 Fast Dx		QSDx	
	InfB	YAM	InfB	YAM	InfB	YAM	InfB	YAM
$10^{3.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)
$10^{1.4}$	0/3 (+)	0/3 (+)	2/3 (+)	2/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	1/3 (+)
$10^{0.7}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-9. 7500 Fast Dx vs. QSDx: Influenza A/gyrfalcon/Washington/41088-6/2014 (H5N8)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme					
	7500 Fast Dx			QSDx		
	InfA	H5a	H5b	InfA	H5a	H5b
$10^{4.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{4.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{3.4}$	2/3 (+)	2/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)	2/3 (+)
$10^{2.7}$	1/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)
$10^{2.0}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-10. 7500 Fast Dx vs. QSDx: Influenza A/gyrfalcon/Washington/41088-6/2014 PR8-IDCDC-RG43A (H5N8)

Titer (EID_{50}/mL)[1]	qScript Enzyme					
	7500 Fast Dx			QSDx		
	InfA	H5a	H5b	InfA	H5a	H5b
$10^{4.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{3.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.1}$	3/3 (+)	2/3 (+)	2/3 (+)	3/3 (+)	3/3 (+)	1/3 (+)
$10^{1.4}$	1/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-11. 7500 Fast Dx vs. QMDx: Influenza A/Hong Kong/4801/2014 (H3N2)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx		QMDx		7500 Fast Dx		QMDx	
	InfA	H3	InfA	H3	InfA	H3	InfA	H3
$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.4}$	3/3 (+)	1/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{0.7}$	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	2/3 (+)
$10^{0.0}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	1/3 (+)	1/3 (+)	1/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-12. 7500 Fast Dx vs. QMDx: Influenza A/Michigan/45/2015 (H1N1)pdm09

Titer (EID_{50}/mL)[1]	SuperScript Enzyme						qScript Enzyme					
	7500 Fast Dx			QMDx			7500 Fast Dx			QMDx		
	InfA	pdm InfA	pdmH1	InfA	pdm InfA	pdmH1	InfA	pdm InfA	pdmH1	InfA	pdm InfA	pdmH1
$10^{2.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.5}$	3/3 (+)	3/3 (+)	1/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	0/3 (+)	2/3 (+)	2/3 (+)	0/3 (+)	0/3 (+)
$10^{0.8}$	0/3 (+)	0/3 (+)	0/3 (+)	2/3 (+)	1/3 (+)	0/3 (+)	2/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)
$10^{0.1}$	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-13. 7500 Fast Dx vs. QMDx: Influenza B/Montana/05/2012 (B/Victoria)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx		QMDx		7500 Fast Dx		QMDx	
	InfB	VIC	InfB	VIC	InfB	VIC	InfB	VIC
$10^{3.0}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.3}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.6}$	3/3 (+)	3/3 (+)	3/3 (+)	1/3 (+)	3/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)
$10^{0.9}$	2/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)
$10^{0.2}$	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-14. 7500 Fast Dx vs. QMDx: Influenza B/Massachusetts/02/2012 (B/Yamagata)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx		QMDx		7500 Fast Dx		QMDx	
	InfB	YAM	InfB	YAM	InfB	YAM	InfB	YAM
$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.4}$	2/3 (+)	3/3 (+)	1/3 (+)	3/3 (+)	0/3 (+)	2/3 (+)	0/3 (+)	3/3 (+)
$10^{0.7}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)
$10^{0.0}$	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Table 8-15. 7500 FastDx vs. QMDx: Influenza A/gyrfalcon/41088-6/2014 PR8-IDCDC-RG43A (H5N8)

Titer (EID_{50}/mL)[1]	SuperScript Enzyme						qScript Enzyme					
	7500 Fast Dx			QMDx			7500 Fast Dx			QMDx		
	InfA	H5a	H5b	InfA	H5a	H5b	InfA	H5a	H5b	InfA	H5a	H5b
$10^{3.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	2/3 (+)
$10^{2.1}$	3/3 (+)	2/3 (+)	3/3 (+)	3/3 (+)	2/3 (+)	3/3 (+)	3/3 (+)	2/3 (+)	2/3 (+)	3/3 (+)	1/3 (+)	1/3 (+)
$10^{1.4}$	2/3 (+)	1/3 (+)	2/3 (+)	1/3 (+)	0/3 (+)	1/3 (+)	3/3 (+)	0/3 (+)	1/3 (+)	2/3 (+)	0/3 (+)	0/3 (+)
$10^{0.7}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)
$10^{0.0}$	0/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)

[1]EID_{50} = Egg Infectious Dose 50%

Analytical Precision – Reproducibility

Studies were performed to assess the reproducibility of the QIAGEN QMDx and Applied Biosystems™ QSDx instruments with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. A blinded panel of contrived influenza A and influenza B samples containing a background of BPL-treated A549 cells in VTM was assembled by adding a BPL-treated influenza A(H3N2) virus, A/Hong Kong/4801/2014, or an influenza B/Victoria virus, B/Nevada/03/2011, respectively. The samples included a moderate positive sample and a low positive sample near the established assay LOD as well as a negative sample consisting of background A549 cells and VTM. Three separate testing sites were selected for testing each PCR instrument. Testing was performed by two different analysts at each site

over five different days. Analysts extracted nucleic acid from the contrived samples and performed rRT-PCR with the InfA, H3, pdmInfA, pdmH1, InfB, YAM, VIC and RP assays from the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel using Invitrogen SuperScript enzyme. The results for the reproducibility study are summarized in Tables 8-16 and 8-17. Both instruments demonstrated high reproducibility with ≥ 93.3% agreement across different sites, analysts, and days.

Table 8-16. Reproducibility Summary –Applied Biosystems™ QSDx instrument

Panel Sample	Primer/ Probe Set	Site 1			Site 2			Site 3			Agreement Total	95% CI
		Agreement	AVG Ct	%CV	Agreement	AVG Ct	%CV	Agreement	AVG Ct	%CV		
sample 1 Moderate A/H3N2	InfA	10/10	27.70	2.46	10/10	29.08	2.53	10/10	29.80	1.25	30/30	100.0 (88.7 - 100.0)
	H3	10/10	28.53	2.96	10/10	29.45	2.98	10/10	30.93	1.27	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7(83.3 - 99.4)
	VIC	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7(83.3 - 99.4)
	YAM	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7(83.3 - 99.4)
	RP	10/10	23.41	6.51	10/10	24.52	3.01	10/10	25.50	1.63	30/30	100.0 (88.7 - 100.0)
sample 2 Moderate B/Victoria	InfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	H3	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	24.70	3.99	10/10	25.54	1.39	10/10	27.17	1.20	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	27.31	8.60	10/10	25.30	3.20	10/10	28.19	2.28	30/30	100.0 (88.7 - 100.0)
	YAM	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7(83.3 - 99.4)
	RP	10/10	23.62	8.17	10/10	24.46	3.46	10/10	25.41	1.57	30/30	100.0 (88.7 - 100.0)
sample 3 Negative	InfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	H3	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	YAM	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7(83.3 - 99.4)
	RP	10/10	25.50	3.50	10/10	26.55	3.91	10/10	28.10	1.11	30/30	100.0 (88.7 - 100.0)
sample 4 Low A/H3N2	InfA	8/10	31.78	1.84	10/10	33.10	2.27	10/10	33.96	1.70	28/30	93.3(78.7 - 98.2)
	H3	10/10	33.18	6.32	10/10	33.75	2.66	10/10	35.01	1.97	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	YAM	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7(83.3 - 99.4)
	RP	10/10	23.05	10.76	10/10	24.76	1.90	10/10	25.75	1.53	30/30	100.0 (88.7 - 100.0)
sample 5 Low B/Victoria	InfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	H3	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	29.41	0.92	10/10	30.26	1.99	10/10	31.73	1.24	30/30	100.0 (88.7 - 100.0)
	VIC	8/10	31.37	6.26	10/10	30.00	1.91	10/10	32.89	2.80	28/30	93.3(78.7 - 98.2)
	YAM	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	RP	10/10	21.98	10.97	10/10	24.46	2.53	10/10	25.75	0.95	30/30	100.0 (88.7 - 100.0)

n/a = not applicable

Table 8-17. Reproducibility Summary –QIAGEN Rotor-Gene Q MDx instrument

Panel Sample	Primer/ Probe Set	Site 1			Site 2			Site 3			Agreement Total	95% CI
		Agreement	AVG Ct	%CV	Agreement	AVG Ct	%CV	Agreement	AVG Ct	%CV		
sample 1 Moderate A/H3N2	InfA	10/10	27.59	1.73	10/10	25.69	1.02	10/10	25.95	0.95	30/30	100.0 (88.7 - 100.0)
	H3	10/10	28.86	2.03	10/10	26.54	1.28	10/10	26.80	0.88	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	YAM	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	RP	10/10	23.74	1.71	10/10	21.88	0.50	10/10	22.21	1.30	30/30	100.0 (88.7 - 100.0)
sample 2 Moderate B/Victoria	InfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	H3	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	26.02	1.46	10/10	24.06	0.69	10/10	24.26	0.85	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	27.06	2.21	10/10	25.17	2.54	10/10	25.36	2.71	30/30	100.0 (88.7 - 100.0)
	YAM	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	RP	10/10	23.73	0.85	10/10	21.92	0.30	10/10	22.14	1.41	30/30	100.0 (88.7 - 100.0)
sample 3 Negative	InfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	H3	9/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	29/30	96.7 (83.3-99.4)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	YAM	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	RP	10/10	26.17	2.96	10/10	23.87	0.98	10/10	24.52	1.21	30/30	100.0 (88.7 - 100.0)
sample 4 Low A/H3N2	InfA	10/10	31.61	1.69	10/10	29.75	1.16	10/10	29.93	1.97	30/30	100.0 (88.7 - 100.0)
	H3	10/10	32.75	2.64	10/10	30.61	2.05	10/10	30.81	0.93	30/30	100.0 (88.7 - 100.0)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	VIC	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	YAM	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	RP	10/10	23.90	1.65	10/10	21.90	0.54	10/10	22.32	1.83	30/30	100.0 (88.7 - 100.0)
sample 5 Low B/Victoria	InfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	H3	10/10	n/a	n/a	10/10	n/a	n/a	9/10	n/a	n/a	29/30	96.7 (83.3-99.4)
	pdmInfA	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	pdmH1	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	InfB	10/10	30.71	4.70	10/10	28.25	1.04	10/10	28.53	1.08	30/30	100.0 (88.7 - 100.0)
	VIC	9/10	32.85	3.41	10/10	29.94	2.08	10/10	30.58	3.52	29/30	96.7 (83.3-99.4)
	YAM	10/10	n/a	n/a	10/10	n/a	n/a	10/10	n/a	n/a	30/30	100.0 (88.7 - 100.0)
	RP	10/10	23.70	0.92	10/10	21.88	0.31	10/10	22.18	1.18	30/30	100.0 (88.7 - 100.0)

n/a = not applicable

Carryover and Cross-contamination Study

The potential for carryover and cross-contamination when testing samples of high viral RNA concentration using either the QIAGEN QMDx or Applied Biosystems™ QSDx instruments with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel was examined. An alternating pattern of high positive and negative contrived samples was analyzed using the InfA, H5a, H5b, and RP assays from the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Contrived samples were prepared using characterized stocks of BPL-inactivated influenza A/gyrfalcon/41088-6/2014 PR8-IDCDC-RG43A (H5N8) in a suspension of A549 cells. A total of 5 individual runs were performed on each instrument. The percent agreement with the expected result was calculated to determine any carryover and cross-contamination effect. The results for each instrument are summarized in Tables 8-18 and 8-19. No carryover or cross–contamination effect was seen with either instrument.

Table 8-18: Carryover and Cross-contamination Study Summary- Applied Biosystems™ QSDx

Assay	Sample[1]	Run 1	Run 2	Run 3	Run 4	Run 5	% Agreement[2]	Sample[1]	Run 1	Run 2	Run 3	Run 4	Run 5	% Agreement[2]
InfA	HP1	18.01	18.88	18.25	19.69	19.33	100.00	N1	0.00	0.00	0.00	0.00	0.00	100.00
	HP2	18.41	18.80	18.28	19.10	18.51	100.00	N2	0.00	0.00	0.00	0.00	0.00	100.00
	HP3	17.83	18.40	17.68	18.65	18.60	100.00	N3	0.00	0.00	0.00	0.00	0.00	100.00
	HP4	18.19	18.92	17.99	18.83	18.28	100.00	N4	0.00	0.00	0.00	0.00	0.00	100.00
	HP5	17.86	18.77	17.73	18.74	18.44	100.00	N5	0.00	0.00	0.00	0.00	0.00	100.00
H5a	HP1	21.14	21.78	21.50	21.91	22.94	100.00	N1	0.00	0.00	0.00	0.00	0.00	100.00
	HP2	21.39	22.07	21.66	22.38	22.07	100.00	N2	0.00	0.00	0.00	0.00	0.00	100.00
	HP3	20.79	21.24	20.54	21.10	22.16	100.00	N3	0.00	0.00	0.00	0.00	0.00	100.00
	HP4	21.04	21.90	20.97	22.08	21.67	100.00	N4	0.00	0.00	0.00	0.00	0.00	100.00
	HP5	20.91	21.33	20.94	21.55	21.99	100.00	N5	0.00	0.00	0.00	0.00	0.00	100.00
H5b	HP1	26.52	27.17	28.86	30.16	31.50	100.00	N1	0.00	0.00	0.00	0.00	0.00	100.00
	HP2	25.91	27.40	28.51	29.40	29.13	100.00	N2	0.00	0.00	0.00	0.00	0.00	100.00
	HP3	25.85	25.86	26.90	27.68	28.55	100.00	N3	0.00	0.00	0.00	0.00	0.00	100.00
	HP4	25.64	27.90	27.11	28.87	28.44	100.00	N4	0.00	0.00	0.00	0.00	0.00	100.00
	HP5	26.19	26.44	26.89	28.07	29.52	100.00	N5	0.00	0.00	0.00	0.00	0.00	100.00
RP	HP1	27.40	28.46	27.47	28.22	28.18	100.00	N1	26.42	27.16	26.66	27.13	27.30	100.00
	HP2	27.71	28.45	27.83	28.40	27.82	100.00	N2	26.11	26.45	25.84	26.59	27.37	100.00
	HP3	27.39	27.96	27.32	27.75	27.86	100.00	N3	26.16	27.05	26.14	26.85	26.97	100.00
	HP4	28.13	28.81	27.64	28.59	27.76	100.00	N4	25.38	25.96	25.36	25.76	27.15	100.00
	HP5	27.52	28.01	27.50	27.97	27.73	100.00	N5	26.21	26.92	25.90	26.90	26.63	100.00

[1]HP = high positive sample; N = negative sample
[2]Percent agreement with the expected result

Table 8-19: Carryover and Cross-contamination Study Summary- QIAGEN Rotor-Gene Q MDx

Assay	Sample[1]	Run 1	Run 2	Run 3	Run 4	Run 5	% Agreement[2]	Sample[1]	Run 1	Run 2	Run 3	Run 4	Run 5	% Agreement[2]
InfA	HP1	17.11	17.09	17.20	20.95	21.89	100.00	N1	0.00	0.00	0.00	0.00	0.00	100.00
	HP2	16.81	16.84	16.87	19.83	18.98	100.00	N2	0.00	0.00	0.00	0.00	0.00	100.00
	HP3	16.77	16.83	16.89	19.76	19.07	100.00	N3	0.00	0.00	0.00	0.00	0.00	100.00
	HP4	16.71	16.73	16.77	19.71	19.61	100.00	N4	0.00	0.00	0.00	0.00	0.00	100.00
	HP5	16.18	16.70	16.73	19.84	19.80	100.00	N5	0.00	0.00	0.00	0.00	0.00	100.00
H5a	HP1	20.94	21.07	21.29	21.65	21.34	100.00	N1	0.00	0.00	0.00	0.00	0.00	100.00
	HP2	20.01	20.55	20.40	20.93	21.23	100.00	N2	0.00	0.00	0.00	0.00	0.00	100.00
	HP3	20.31	20.42	20.85	21.16	21.12	100.00	N3	0.00	0.00	0.00	0.00	0.00	100.00
	HP4	20.26	20.52	20.26	20.65	20.32	100.00	N4	0.00	0.00	0.00	0.00	0.00	100.00
	HP5	20.22	20.28	20.29	20.44	20.87	100.00	N5	0.00	0.00	0.00	0.00	0.00	100.00
H5b	HP1	27.20	29.72	26.99	29.46	28.22	100.00	N1	0.00	0.00	0.00	0.00	0.00	100.00
	HP2	28.32	28.52	27.23	27.08	25.76	100.00	N2	0.00	0.00	0.00	0.00	0.00	100.00
	HP3	26.28	28.72	26.33	28.13	25.10	100.00	N3	0.00	0.00	0.00	0.00	0.00	100.00
	HP4	25.13	29.10	26.05	26.94	24.60	100.00	N4	0.00	0.00	0.00	0.00	0.00	100.00
	HP5	25.54	26.66	24.57	26.12	25.28	100.00	N5	0.00	0.00	0.00	0.00	0.00	100.00
RP	HP1	26.41	26.61	26.43	26.67	26.57	100.00	N1	25.46	25.66	25.59	25.79	25.66	100.00
	HP2	26.52	26.51	26.56	26.51	26.66	100.00	N2	25.65	25.83	25.66	25.67	25.84	100.00
	HP3	26.32	25.89	26.09	26.51	26.09	100.00	N3	25.46	25.65	25.62	25.48	25.48	100.00
	HP4	26.41	26.48	26.46	26.64	26.48	100.00	N4	25.37	25.56	25.47	25.53	25.53	100.00
	HP5	25.89	26.36	25.93	26.02	26.45	100.00	N5	25.55	24.78	25.01	24.90	25.87	100.00

[1]HP = high positive sample; N = negative sample

[2]Percent agreement with the expected result

IX. CLINICAL PERFORMANCE EVALUATION

The clinical performance of the Applied Biosystems™ QSDx and QIAGEN Rotor-Gene Q MDx instruments was evaluated to demonstrate equivalency with the FDA-cleared Applied Biosystems 7500 Fast Dx when using the CDC Human Influenza Real-Time Diagnostic Panel. The study was performed using retrospective clinical specimens collected during the 2013-2014 influenza seasons. The lack of available clinical specimens containing influenza A(H5) viruses was addressed using ten contrived samples prepared with BPL-inactivated influenza A(H5) virus in a suspension of human A549 cells and virus transport medium. A total of 50 clinical specimens and contrived samples that were previously determined to be positive using the Applied Biosystems™ 7500 Fast Dx for influenza A(H1)pdm09, A(H3), A(H5), B/Victoria, or B/Yamagata virus and 50 negative specimens were evaluated with the CDC Human Influenza Real-Time Diagnostic Panel. Samples were extracted using the Roche MagNA Pure Compact and RNA Isolation Kit. Testing was performed using Invitrogen SuperScript enzyme mastermix and utilizing either the Applied Biosystems™ QSDx or QIAGEN QMDx. The results are summarized in the Tables 8-20 to 8-21. Each instrument demonstrated 100% agreement with the comparator.

Table 8-20. Retrospective Clinical Results- Applied Biosystems™ QSDx

	7500 Fast Dx				
QSDx	Positive	Negative	Total	Percent Agreement	95% CI
Positive	50	0	50	100	92.9-100.0
Negative	0	50	50	100	92.9-100.0
Total	50	50			

Table 8-21. Retrospective Clinical Results- QIAGEN Rotor-Gene QMDx

	7500 Fast Dx				
QMDx	Positive	Negative	Total	Percent Agreement	95% CI
Positive	50	0	50	100	92.9-100.0
Negative	0	50	50	100	92.9-100.0
Total	50	50			

X. CONCLUSION

Performance studies were conducted to evaluate the modification of the CDC Human Influenza Virus rRT-PCR Diagnostic Panel to add real-time PCR instrument options that are acceptable for use with the CDC device. Evaluation of the LOD equivalency, reproducibility, carryover and cross-contamination, and testing of clinical samples demonstrated that the modified device is substantially equivalent to the predicate.