510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K190302

B. Purpose for Submission:
Device modification to add two additional real-time PCR instrument options, the Applied Biosystems QuantStudio Dx (QSDx) and the QIAGEN Rotor-Gene Q MDx (QMDx), that are acceptable for use with the following previsouly FDA-cleared diagnostic kits, the 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), Influenza B Lineage Genotyping Kit (VER 2), and Influenza A/H5 Subtyping Kit (VER 3).

C. Measurand:
Influenza A, Influenza B, seasonal human influenza A(H3), seasonal human influenza A(H1)pdm09, B/Victoria genetic lineage of human influenza B, B/Yamagata genetic lineage of human influenza B, and influenza A subtype A(H5) (Asian lineage) viral RNA target sequences.

D. Type of Test:
Real-time RT-PCR (rRT-PCR) assays.

E. Applicant:
Centers for Disease Control and Prevention.

F. Proprietary and Established Names:
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).

G. Regulatory Information:

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

Product Code	Classification	Regulation Section	Panel
OZE	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
NSU	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

OOI	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel - Influenza A Subtyping Kit (VER 2)

Product Code	Classification	Regulation Section	Panel
OZE	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OEP	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OQW	Class II	21 CFR 866.3332 Reagents for Detection of Specific Novel Influenza A Viruses	Microbiology (83)
NSU	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)
OOI	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

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

Product Code	Classification	Regulation Section	Panel
OZE	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
NSU	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)
OOI	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

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

Product Code	Classification	Regulation Section	Panel
OZE	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OEP	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
NXD	Class II	21 CFR 866.3332 Reagents for Detection of Specific Novel Influenza A Viruses	Microbiology (83)
NSU	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)
OOI	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:
   Same as Intended Use(s)

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   - 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 (US) Plug-in version 1.0.1 software

I. 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 instrument that has been FDA-cleared for use with this device. 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 are also included in the panel.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   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)

2. Predicate 510(k) number(s):
   K172091
   K181736

3. Comparison with predicate:

Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/B Typing Kit (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/B Typing Kit (K190302)**
Specimen Types	Upper respiratory tract and lower respiratory tract specimens	Same
Organisms Detected	Influenza A viruses (animal and human) and influenza B viruses	Same
Analyte	RNA	Same
Technological Principles	Real-time RT-PCR	Same
Instrumentation	Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4	• Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4 • Applied Biosystems QuantStudio Dx with version 1.0.3 software • QIAGEN Rotor-Gene Q MDx with AssayManager 1.0.4.1 and Epsilon version 1.0.1 software
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
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,	• 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 • 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	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
Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit (VER 2) (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit (VER 2) (K190302)**
Specimen Types	Upper respiratory tract and lower respiratory tract specimens	Same
Organisms Detected	Influenza A viruses (animal and human), swine-origin influenza A viruses, influenza A subtypes: seasonal A(H3) and A(H1)pdm09	Same
Analyte	RNA	Same
Technological Principles	Real-time RT-PCR	Same
Instrumentation	Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4	• Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4 • Applied Biosystems QuantStudio Dx with version 1.0.3 software • QIAGEN Rotor-Gene Q MDx with AssayManager 1.0.4.1 and Epsilon version 1.0.1 software
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
Nucleic Acid Extraction	• QIAamp DSP Viral RNA Mini Kit, QIAGEN • MagNA Pure Compact – Nucleic Acid Isolation Kit I, Roche • MagNA Pure Compact –	• 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 • 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	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
Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza B Lineage Genotyping Kit (VER 1.1 or VER 2) (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza B Lineage Genotyping Kit (VER 1.1 or VER 2) (K190302)**
Specimen Types	Upper respiratory tract and lower respiratory tract specimens	Same
Organisms Detected	Influenza B viruses, lineages B/Victoria and B/Yamagada	Same
Analyte	RNA	Same
Technological Principles	Real-time RT-PCR	Same
Instrumentation	Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4	• Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4 • Applied Biosystems QuantStudio Dx with version 1.0.3 software • QIAGEN Rotor-Gene Q MDx with AssayManager 1.0.4.1 and Epsilon version 1.0.1 software
Enzyme Master Mix	Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR Kit (with or without ROX) or Quanta BioSciences qScript One-Step qRT-	Same

	PCR Kit, Low ROX	
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 • 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
Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/H5 Subtyping Kit (VER 3) (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/H5 Subtyping Kit (VER 3) (K190302)**
Specimen Types	Upper respiratory tract and lower respiratory tract specimens	Same
Organisms Detected	Influenza A viruses (animal and human), and influenza A subtype A(H5) (Asian lineage)	Same
Analyte	RNA	Same
Technological Principles	Real-time RT-PCR	Same
Instrumentation	Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4	• Applied Biosystems 7500 Fast Dx Real-time PCR system with SDS software version 1.4 • Applied Biosystems QuantStudio Dx with version

		1.0.3 software • QIAGEN Rotor-Gene Q MDx with AssayManager 1.0.4.1 and Epsilon version 1.0.1 software
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
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 • 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

* bioMérieux EMAG is a higher throughput robotic system that uses identical chemistry, reagents, and workflow as the bioMérieux NucliSENS easyMAG. CDC internal validation data demonstrated functional performance equivalency of the EMAG to the NucliSENS easyMAG.

K. Standard/Guidance Document Referenced (if applicable):

NA

L. Test Principle:

The CDC Human Influenza Real-Time RT-PCR Diagnostic Panel includes sets of oligonucleotide primers and dual-labeled hydrolysis probes to be used in real-time RT-PCR assays on *in vitro* diagnostic real-time PCR instrument that has been FDA-cleared for use with this device. The targeted regions of influenza viral RNA are transcribed into complimentary DNA (cDNA) and amplified by the polymerase chain reaction (PCR). The fluorescently

labeled probes anneal to amplified DNA fragments and the fluorescent signal intensity is monitored by the real-time PCR instrument during each PCR cycle. Amplification of target is recorded as increase of fluorescence over time in comparison to background signal.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Studies were conducted to assess the reproducibility of the Applied Biosystems QuantStudio Dx (QSDS) and the QIAGEN Rotor-Gene Q MDx (QMDX) 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 beta-propiolactone (BPL) treated A549 cells in viral transport medium (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, a low positive sample near the established assay LoD, and a negative sample consisting of background A549 cells and VTM.

Three separate testing sites were selected for each real-time PCR instrument platform. The contrived sample panel was tested five times by each of the two analysts at each site over five days. Analysts extracted nucleic acid from the contrived samples using a nucleic acid extraction method that has been FDA-cleared for use with this device 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 studies of each real-time PCR instrument platform are summarized in Table 1 and Table 2 below.

Table 1: Reproducibility Study Summary Results – Applied Biosystems QuantStudio Dx (QSDS)

Panel Sample	Assay	Site 1			Site 2			Site 3			Agreement Total	95% CI
		Agreement	Ave. Ct	% CV	Agreement	Ave. Ct	% CV	Agreement	Ave. Ct	% CV		
A/H3N2 Moderate	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)
A/H3N2 Low	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)
B/Victoria Moderate	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)
B/Victoria Low	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)
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)

Table 2: Reproducibility Study Summary Results – QIAGEN Rotor-Gene Q MDx (QMDX)

Panel Sample	Assay	Site 1			Site 2			Site 3			Agreement Total	95% CI
		Agreement	Ave. Ct	% CV	Agreement	Ave. Ct	% CV	Agreement	Ave. Ct	% CV		
A/H3N2 Moderate	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)
A/H3N2 Low	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)
B/Victoria Moderate	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)
B/Victoria Low	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	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)
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	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	26.17	2.96	10/10	23.87	0.98	10/10	24.52	1.21	30/30	100.0 (88.7-100.0)

Each real-time PCR instrument platform demonstrated acceptable reproducibility with ≥ 93.3% agreement across different sites, analysts, and days.

b. Linearity/assay reportable range:

Not applicable, qualitative tests.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Not applicable, aspects of the tests other than the two additional real-time PCR instruments and one additional nucleic acid extraction option are not modified from the previously FDA-cleared versions.

d. Detection limit:

The LoD performance 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 QIAGEN Rotor-Gene Q MDx (QMDx) instrumentwas was evaluated by testing 5-fold serial dilutions of the following five characterized influenza viruses of known egginfectious dose 50% titer: Influenza A/Hong Kong/4801/2014 (A/H3N2), Influenza A/Michigan/45/2015 (A/H1N1pdm09), Influenza B/Montana/05/2012 (B/Victoria), Influenza B/Massachusetts/02/2012 (B/Yamagata), and Influenza A/gyrfalcon/Washington/41088-6/2014 PR8-IDCDC-RG43A (A/H5N8).

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 both theInvitrogen SuperScript III Platinum One-Step RT-PCR System (SuperScript) and the QuantaqScript (qScript) enzyme system. The acceptance criterion for LoD equivalence between the FDA-cleared Applied Biosystems 7500 Fast Dx and the investigational Applied Biosystems QSDx or QIAGEN QMDx instrument was pre-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 strain tested in Tables 3 to 12 below. The lowest concentration at which each assay showed 100% positivity is highlighted green.

Each investigational real-time instrument met the acceptance criterion when compared to the FDA-cleared real-time PCR instrument.

Table 3: LoD Equivalency Determination – 7500 Fast Dx vs. QSDx (Influenza A/Hong Kong/4801/2014 (A/H3N2))

Titer (EID50/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx (Comparator)		QSDx		7500 Fast Dx (Comparator)		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 4: LoD Equivalency Determination – 7500 Fast Dx vs. QSDx (Influenza A/Michigan/45/2015 (A/H1N1pdm09))

Titer (EID50/mL)[1]	SuperScript Enzyme						qScript Enzyme					
	7500 Fast Dx (Comparator)			QSDx			7500 Fast Dx (Comparator)			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 5: LoD Equivalency Determination – 7500 Fast Dx vs. QSDx (Influenza B/Montana/05/2012 (B/Victoria))

Titer (EID50/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx (Comparator)		QSDx		7500 Fast Dx (Comparator)		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 6: LoD Equivalency Determination – 7500 Fast Dx vs. QSDx (Influenza B/Massachusetts/02/2012 (B/Yamagata))

Titer (EID50/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx (Comparator)		QSDx		7500 Fast Dx (Comparator)		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 7: LoD Equivalency Determination – 7500 Fast Dx vs. QSDx (Influenza A/gyrfalcon/Washington/41088-6/2014 PR8-IDCDC-RG43A (A/H5N8))

Titer (EID50/mL)[1]	SuperScript Enzyme						Titer (EID50/mL)[1]	qScript Enzyme					
	7500 Fast Dx (Comparator)			QSDx				7500 Fast Dx (Comparator)			QSDx		
	InfA	H5a	H5b	InfA	H5a	H5b		InfA	H5a	H5b	InfA	H5a	H5b
$10^{4.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	$10^{4.2}$	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 (+)	$10^{3.5}$	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 (+)	$10^{2.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.7}$	2/3 (+)	1/3 (+)	1/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	$10^{2.1}$	3/3 (+)	2/3 (+)	2/3 (+)	3/3 (+)	3/3 (+)	1/3 (+)
$10^{2.0}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	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: LoD Equivalency Determination – 7500 Fast Dx vs. QMDx (Influenza A/Hong Kong/4801/2014 (A/H3N2))

Titer (EID50/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx (Comparator)		QMDx		7500 Fast Dx (Comparator)		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 9: LoD Equivalency Determination – 7500 Fast Dx vs. QMDx (Influenza A/Michigan/45/2015 (A/H1N1pdm09))

Titer (EID50/mL)[1]	SuperScript Enzyme						qScript Enzyme					
	7500 Fast Dx (Comparator)			QMDx			7500 Fast Dx (Comparator)			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 10: LoD Equivalency Determination – 7500 Fast Dx vs. QMDx (Influenza B/Montana/05/2012 (B/Victoria))

Titer (EID50/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx (Comparator)		QMDx		7500 Fast Dx (Comparator)		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 11: LoD Equivalency Determination – 7500 Fast Dx vs. QMDx (Influenza B/Massachusetts/02/2012 (B/Yamagata))

Titer (EID50/mL)[1]	SuperScript Enzyme				qScript Enzyme			
	7500 Fast Dx (Comparator)		QMDx		7500 Fast Dx (Comparator)		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 12: LoD Equivalency Determination – 7500 Fast Dx vs. QMDx (Influenza A/gyrfalcon/Washington/41088-6/2014 PR8-IDCDC-RG43A (A/H5N8))

Titer (EID50/mL)[1]	SuperScript Enzyme						Titer (EID50/mL)[1]	qScript Enzyme					
	7500 Fast Dx (Comparator)			QMDx				7500 Fast Dx (Comparator)			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 (+)	$10^{3.5}$	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 (+)	$10^{2.8}$	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 (+)	$10^{2.1}$	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 (+)	$10^{1.4}$	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 (+)	$10^{0.7}$	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 (+)	$10^{0.0}$	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)	0/3 (+)

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

e. Analytical specificity:

Not applicable, aspects of the tests other than real-time PCR instrument platform and nucleic acid extraction options are not modified from the previously FDA-cleared versions.

f. Assay cut-off:

Not applicable, aspects of the tests other than real-time PCR instrument platform and nucleic acid extraction options are not modified from the previously FDA-cleared versions.

g. Carryover and Cross-contamination:

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 five individual runs were performed on each instrument. The percent agreements with the expected result were calculated to determine any carryover and cross-contamination effect. No carryover or cross–contamination effect was observed with either real-time PCR instrument during this study.

2. Comparison studies:

a. Method comparison with predicate device:

Refer to the "Clinical studies" section

b. Matrix comparison:

Not applicable

3. Clinical studies:

Performance testing clinical specimens using the Applied Biosystems QSDx and the QIAGEN Rotor-Gene Q MDx instrument platforms with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel test kits was evaluated to demonstrate equivalency with the FDA-cleared Applied Biosystems 7500 Fast Dx. The study was performed using retrospective natural clinical specimens collected during the 2013-2014 influenza seasons. The lack of available natural 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 natural clinical specimens and contrived samples that were previously determined to be positive using the Applied Biosystems 7500 Fast Dx (the comparator) for influenza A/H1pdm09 (10 specimens), A/H3 (10 specimens), A/H5 (10 specimens), B/Victoria (10 specimens), or B/Yamagata (10 specimens) and 50 influenza negative natural clinical specimens were evaluated using 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 Table 13 and Table 14 below.
Each real-time PCR instruments demonstrated 100% agreement with the comparator.

Table 13: Retrospective Clinical Specimens and Contrived Specimens Testing Results – Applied Biosystems QSDx

	7500 Fast Dx (Comparator)		Total
QSDx	Positive	Negative	
Positive	50	0	50
Negative	0	50	50
Total	30	50	100
Positive Percent Agreement (PPA) = 100% (50/50), 95% CI: 92.9% to 100%			
Negative Percent Agreement (NPA) = 100% (50/50), 95% CI: 92.9% to 100%			

Table 14: Retrospective Clinical Specimens and Contrived Specimens Testing Results - QIAGEN QMDx

	7500 Fast Dx (Comparator)		Total
QMDx	Positive	Negative	
Positive	50	0	50
Negative	0	50	50
Total	50	50	100
Positive Percent Agreement (PPA) = 100% (50/50), 95% CI: 92.9% to 100%			
Negative Percent Agreement (NPA) = 100% (50/50), 95% CI: 92.9% to 100%			

4. Clinical cut-off**:**

   Not applicable, aspects of the tests other than real-time PCR instrument platform and nucleic acid extraction options are not modified from the previously FDA-cleared versions.

5. Expected values/Reference range:

   Not applicable, aspects of the tests other than real-time PCR instrument platform and nucleic acid extraction options are not modified from the previously FDA-cleared versions.

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.