510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K172091

B. Purpose for Submission:
Device modification to add three additional options of nucleic acids extraction instrument and extraction method combinations (QIAGEN EZ1 DSP Virus Kit on the QIAGEN EZ1 Advanced XL instrument, QIAGEN EZ1 RNA Tissue Mini Kit on the QIAGEN EZ1 Advanced XL instrument, and Roche DNA and Viral NA Small Volume Kit on the Roche MagNA Pure 96 instrument) that are acceptable for use with the four 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, 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, 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

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 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.

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 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.

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:

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.

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 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. 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 (ABI) 7500 Fast Dx Real-Time PCR instrument

I. Device Description:

The CDC Human Influenza Real-Time RT-PCR Diagnostic Panel is used in real-time RT-PCR (rRT-PCR) assays on the Applied Biosystems (ABI) 7500 Fast Dx 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 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
   Influenza A/H5 Subtyping Kit (VER 3)

2. Predicate 510(k) number(s):
   K133869
   K161556
   K140857
   K153148

3. Comparison with predicate:

Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/B Typing Kit (K133869)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/B Typing Kit (K172091)**
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 (ABI) 7500 Fast Dx Real-time PCR system	Same
Enzyme Master Mix	Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR Kit (with or without ROX) or Quanta BioSciences qScript One-Step qRT-PCR Kit, Low ROX	Same
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	• 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

Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit (VER 2) (K161556)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit (VER 2) (K172091)**
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 (ABI) 7500 Fast Dx Real-time PCR system	Same
Enzyme Master Mix	Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR Kit (with or without ROX) or Quanta BioSciences qScript One-Step qRT-PCR Kit, Low ROX	Same
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	• 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

Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza B Lineage Genotyping Kit (K140857)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza B Lineage Genotyping Kit (K172091)**
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 (ABI) 7500 Fast Dx Real-time PCR system	Same
Enzyme Master Mix	Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR Kit (with or without ROX) or Quanta BioSciences qScript One-Step qRT-PCR Kit, Low ROX	Same
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	• 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

Similarities or Differences		
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/H5 Subtyping Kit (VER 3) (K153148)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A/H5 Subtyping Kit (VER 3) (K172091)**
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 (ABI) 7500 Fast Dx Real-time PCR system	Same
Enzyme Master Mix	Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR Kit (with or without ROX) or Quanta BioSciences qScript One-Step qRT-PCR Kit, Low ROX	Same
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	• 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

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 the Applied Biosystems (ABI) 7500 Fast Dx Real-time PCR instrument. 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 ABI 7500 Fast Dx 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:

Using the CDC Influenza A Subtyping Kit as the representative assays from the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel, studies were conducted to assess the reproducibility of the two additional nucleic acids extraction instrument platforms to be used with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel test kits. The Roche MagNA Pure 96 was evaluated with the Roche DNA and Viral NA Small Volume Kit. The QIAGEN EZ1 Advanced XL was evaluated with the QIAGEN EZ1 DSP Virus Kit and the QIAGEN EZ1 RNA Tissue Mini Kit.

A blinded panel of contrived 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. The samples included a moderate positive sample, a low positive sample near the established assay LoD for the CDC Influenza A Subtyping Kit, and a negative sample consisting of background A549 cells and VTM.

Three separate testing sites were selected for each extraction instrument platform. The sample panel was tested five times by two analysts at each site over five days. Analysts performed extractions with the investigational nucleic acids extraction instrument platform and the relevant extraction method(s),

and tested the extracted nucleic acids with the InfA, H3, and RP assays from the CDC Influenza A Subtyping Kit using Invitrogen SuperScript and utilizing the Applied Biosystems 7500 Fast Dx (ABI 7500 Fast Dx) real-time PCR system.

The results for the reproducibility studies of each nucleic acids extraction instrument platform and the relevant extraction method(s) are summarized in Table 1 to Table 3 below.

Table 1: Reproducibility Study Summary Results – QIAGEN EZ1 Advanced XL, EZ1 DSP Virus Kit

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(H3) Moderate	InfA	10/10	25.46	2.36	10/10	26.45	2.80	10/10	28.46	4.12	30/30	100.0 (88.6-100.0)
	H3	10/10	27.15	2.92	10/10	28.22	3.25	10/10	28.61	4.40	30/30	100.0 (88.6-100.0)
	RP	10/10	22.45	1.44	10/10	23.47	2.42	10/10	24.50	5.68	30/30	100.0 (88.6-100.0)
A(H3) Low	InfA	10/10	28.96	3.41	10/10	30.35	1.87	10/10	32.21	2.55	30/30	100.0 (88.6-100.0)
	H3	10/10	30.56	2.60	10/10	31.70	1.96	10/10	32.63	3.18	30/30	100.0 (88.6-100.0)
	RP	10/10	22.26	1.08	10/10	23.53	1.12	10/10	24.58	4.63	30/30	100.0 (88.6-100.0)
Negative	InfA	10/10	0.00	n/a	10/10	0.00	n/a	10/10	0.00	n/a	30/30	100.0 (88.6-100.0)
	H3	10/10	0.00	n/a	10/10	0.00	n/a	10/10	0.00	n/a	30/30	100.0 (88.6-100.0)
	RP	10/10	24.80	1.49	10/10	25.35	3.91	10/10	26.93	4.83	30/30	100.0 (88.6-100.0)

Table 2: Reproducibility Study Summary Results – QIAGEN EZ1 Advanced XL, EZ1 RNA Tissue Mini Kit

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(H3) Moderate	InfA	10/10	25.97	3.48	10/10	26.40	4.06	10/10	28.59	2.01	30/30	100.0 (88.6-100.0)
	H3	10/10	27.40	4.13	10/10	27.56	2.35	10/10	28.66	0.79	30/30	100.0 (88.6-100.0)
	RP	10/10	22.08	3.31	10/10	23.52	1.56	10/10	24.77	2.14	30/30	100.0 (88.6-100.0)
A(H3) Low	InfA	10/10	30.07	2.04	10/10	30.30	3.26	10/10	32.37	2.44	30/30	100.0 (88.6-100.0)
	H3	10/10	31.02	3.14	10/10	30.94	2.06	10/10	32.59	1.15	30/30	100.0 (88.6-100.0)
	RP	10/10	21.88	2.37	10/10	23.29	2.32	10/10	24.30	2.93	30/30	100.0 (88.6-100.0)
Negative	InfA	10/10	0.00	n/a	10/10	0.00	n/a	10/10	0.00	n/a	30/30	100.0 (88.6-100.0)
	H3	10/10	0.00	n/a	10/10	0.00	n/a	10/10	0.00	n/a	30/30	100.0 (88.6-100.0)
	RP	10/10	24.36	2.57	10/10	25.32	1.55	10/10	27.38	1.85	30/30	100.0 (88.6-100.0)

Table 3: Reproducibility Study Summary Results – Roche MagNA Pure 96, DNA and Viral NA Small Volume Kit

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(H3) Moderate	InfA	10/10	31.29	3.97	10/10	30.05	3.37	10/10	27.61	3.93	30/30	100.0 (88.6-100.0)
	H3	10/10	32.00	3.36	10/10	31.92	3.32	10/10	28.44	4.05	30/30	100.0 (88.6-100.0)
	RP	10/10	27.16	4.07	10/10	24.25	1.66	10/10	23.35	3.48	30/30	100.0 (88.6-100.0)
A(H3) Low	InfA	10/10	34.91	2.97	10/10	33.49	2.86	10/10	31.40	3.07	30/30	100.0 (88.6-100.0)
	H3	10/10	35.45	2.54	10/10	35.35	2.75	10/10	32.37	3.30	30/30	100.0 (88.6-100.0)
	RP	10/10	26.97	3.85	10/10	24.22	0.98	10/10	23.11	2.70	30/30	100.0 (88.6-100.0)
Negative	InfA	10/10	0.00	n/a	10/10	0.00	n/a	10/10	0.00	n/a	30/30	100.0 (88.6-100.0)
	H3	10/10	0.00	n/a	10/10	0.00	n/a	10/10	0.00	n/a	30/30	100.0 (88.6-100.0)
	RP	10/10	29.40	3.66	10/10	26.64	1.36	10/10	25.58	2.91	30/30	100.0 (88.6-100.0)

Each nucleic acids extraction instrument platform and the relevant extraction

method demonstrated acceptable reproducibility with 100% 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 nucleic acids extraction options are not modified from the previously FDA-cleared versions.

d. *Detection limit:*

The LoD performance equivalency between a previously FDA-cleared nucleic acids extraction method and either the Roche MagNA Pure 96 or the QIAGEN EZ1 Advanced XL nucleic acids extraction instrument platform and the relevant extraction method was evaluated by testing 5-fold serial dilutions of a characterized influenza A(H3N2) virus, A/Hong Kong/4801/2014, of known embrio infectious dose 50% titer (EID_{50}), using the CDC Influenza A Subtyping Kit as the representative assays from the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel.

Virus dilutions were prepared using a suspension of BPL treated A549 cells in VTM as diluent. Triplicate samples of each dilution were extracted separately with the previously FDA-cleared Roche MagNA Pure Compact RNA Isolation Kit, as well as the investigational nucleic acids extraction instrument platform and the relevant extraction method. The Roche MagNA Pure 96 was evaluated with the Roche DNA and Viral NA Small Volume Kit. The QIAGEN EZ1 Advanced XL was evaluated with the QIAGEN EZ1DSP Virus Kit and the QIAGEN EZ1 RNA Tissue Mini Kit.

Extracted RNA was tested with the InfA and H3 assays from the CDC Influenza A Subtyping Kit using Invitrogen SuperScript III Platinum One-Step RT-PCR System (Invitrogen SuperScript™) and utilizing the ABI 7500 Fast Dx real-time PCR system.

The pre-established acceptance criteria for demonstrating LoD equivalence between the previously FDA-cleared and the investigational extraction methods 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 in Table 4 to Table 6 below.

Table 4: LoD Equivalency Determination – QIAGEN EZ1 Advanced XL, EZ1 DSP Virus Kit

Titer (EID_{50}/mL)[1]	Roche MagNA Pure Compact - RNA Isolation Kit (Comparator)		QIAGEN EZ1 Advanced XL – EZ1 DSP Virus Kit	
	InfA	H3	InfA	H3
$10^{3.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.3}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{0.4}$	1/3 (+)	0/3 (+)	1/3 (+)	0/3 (+)

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

Table 5: LoD Equivalency Determination – QIAGEN EZ1 Advanced XL, EZ1 RNA Tissue Mini Kit

Titer (EID_{50}/mL)[1]	Roche MagNA Pure Compact - RNA Isolation Kit (Comparator)		QIAGEN EZ1 Advanced XL – EZ1 RNA Tissue Mini Kit	
	InfA	H3	InfA	H3
$10^{3.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.3}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{0.4}$	1/3 (+)	0/3 (+)	2/3 (+)	2/3 (+)

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

Table 6: LoD Equivalency Determination - Roche MagNA Pure 96, DNA and Viral NA Small Volume Kit

Titer (EID_{50}/mL)[1]	Roche MagNA Pure Compact - RNA Isolation Kit (Comparator)		Roche MagNA Pure 96 - DNA and Viral NA Small Volume Kit	
	InfA	H3	InfA	H3
$10^{3.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.3}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{0.4}$	1/3 (+)	0/3 (+)	2/3 (+)	2/3 (+)

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

Each of the three investigational nucleic acids extraction instrument platform and extraction method combinations demonstrated an equivalent endpoint LoD concentration when compared to the previously FDA-cleared nucleic acids extraction instrument platform and extraction method combination (comparator).

e. Analytical specificity:

Not applicable, aspects of the tests other than nucleic acids extraction options are not modified from the previously FDA-cleared versions.

f. Assay cut-off:

Not applicable, aspects of the tests other than nucleic acids extraction options are not modified from the previously FDA-cleared versions.

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 natural clinical specimens using the two additional nucleic acids extraction instrument platforms with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel test kits, the Roche MagNA Pure 96 and the QIAGEN EZ1 Advanced XL instrument platforms, was evaluated in a study using the CDC Influenza A Subtyping Kit as the representative assays from the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel to demonstrate equivalent performance when testing natural clinical specimens to a nucleic acids extraction instrument platform and extraction method combination that was previously FDA-cleared for use with the CDC Human Influenza Real-Time Diagnostic Panel test kits (the comparator).

The Roche MagNA Pure 96 was evaluated using the Roche DNA and Viral NA Small Volume Kit. The QIAGEN EZ1 Advanced XL was evaluated using the QIAGEN EZ1 DSP Virus Kit and the QIAGEN EZ1 RNA Tissue Mini Kit. The comparator was the Roche MagNA Pure Compact instrument using the RNA Isolation Kit.

The study was conducted internally testing a set of retrospective clinical specimens collected during the 2011-2012 and 2013-2014 influenza seasons. A total of thirty clinical specimens that were previously determined to be positive for influenza A(H3) virus and thirty negative clinical specimens were evaluated with the InfA, H3, and RP assays from the CDC Influenza A Subtyping Kit as representative assays from the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel. Testing was performed using Invitrogen SuperScript and utilizing the ABI 7500 Fast Dx real-time PCR system. The study results are summarized in the Table 7 to Table 9 below.

Table 7: Retrospective Clinical Study Results - QIAGEN EZ1 Advanced XL, EZ1 DSP Virus Kit

	Roche MagNA Pure Compact – RNA Isolation Kit (Comparator)		Total
QIAGEN Advanced XL – EZ1 DSP Virus Kit	Positive	Negative	
Positive	30	0	30
Negative	0	30	30
Total	30	30	60
Positive Percent Agreement (PPA) = 100% (30/30), 95% CI: 88.6% to 100%			
Negative Percent Agreement (NPA) = 100% (30/30), 95% CI: 88.6% to 100%			

Table 8: Retrospective Clinical Study Results - QIAGEN EZ1 Advanced XL, EZ1 RNA Tissue Mini Kit

	Roche MagNA Pure Compact – RNA Isolation Kit (Comparator)		Total
QIAGEN Advanced XL – EZ1 RNA Tissue Mini Kit	Positive	Negative	
Positive	30	0	30
Negative	0	30	30
Total	30	30	60
Positive Percent Agreement (PPA) = 100% (30/30), 95% CI: 88.6% to 100%			
Negative Percent Agreement (NPA) = 100% (30/30), 95% CI: 88.6% to 100%			

Table 9: Retrospective Clinical Study Results – Roche MagNA Pure 96, DNA and Viral NA Small Volume Kit

	Roche MagNA Pure Compact – RNA Isolation Kit (Comparator)		Total
Roche MagNA Pure 96 – DNA and Viral NA Small Volume Kit	Positive	Negative	
Positive	30	0	30
Negative	0	30	30
Total	30	30	60
Positive Percent Agreement (PPA) = 100% (30/30), 95% CI: 88.6% to 100%			
Negative Percent Agreement (NPA) = 100% (30/30), 95% CI: 88.6% to 100%			

Each of the three investigational nucleic acids extraction instrument platform and extraction method combinations demonstrated 100% agreement with the comparator.

4. Clinical cut-off**:**

   Not applicable, aspects of the tests other than nucleic acids extraction options are not modified from the previously FDA-cleared versions.

5. Expected values/Reference range:

   Not applicable, aspects of the tests other than nucleic acids extraction options are not modified from the previously FDA-cleared versions.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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