DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
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June 30, 2016

Centers for Disease Control and Prevention (CDC)
Yon Yu, Pharm.D.
Associate Director for Regulatory Affairs
1600 Clifton Road, MS-C18
Atlanta, GA 30329-4027

Re: K161556
Trade/Device Name: CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: II
Product Code: OZE, NSU, NXD, OEP, OQW
Dated: June 3, 2016
Received: June 6, 2016

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. 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 Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. 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 the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K161556

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

Indications for Use *(Describe)*
The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an 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.

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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Contact Person:
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Date Prepared: June 3, 2016

II. DEVICE INFORMATION

Proprietary Name:	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (VER 2)
Common Name:	Influenza A 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
Panel:	Microbiology

III. PREDICATE DEVICE

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (K140851)

IV. DEVICE DESCRIPTION

The CDC Human Influenza Real-Time RT-PCR Diagnostic Panel Influenza A Subtyping Kit is a real-time RT-PCR (rRT-PCR) assay that utilizes the Applied Biosystems® (ABI) 7500 Fast Dx Real-time PCR system. The Influenza A Subtyping 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). The oligonucleotide primers and probes for detection of influenza A and 2009 influenza A (swine origin) were selected from highly conserved regions of the matrix (M), and the nucleoprotein (NP), respectively. Oligonucleotide primers and probes for characterization and differentiation of influenza A(H3) and A(H1)pdm09 viruses were selected from highly conserved regions of their HA genes. The Influenza A Subtyping kit also contains primers and probes to detect the human RNase P gene (RP) in control samples and clinical specimens.

V. INTENDED USE

The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an 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.

VI. TECHNOLOGICAL CHARACTERISTICS

CDC has identified nucleotide substitutions in the HA gene segment in a small proportion of currently circulating influenza A(H1N1)pdm09 viruses belonging to the 6B.1 genetic clade. The nucleotide substitutions are located in the targeted regions of the reverse primer and the probe of the pdmH1 assay and may cause aberrant reactivity of the pdmH1 assay included in the Influenza A Subtyping Kit. CDC is modifying the pdmH1 assay to improve reactivity with the 6B.1 clade. The performance of the remaining assays in the Influenza A Subtyping Kit (InfA, H3, pdmInfA, and RP) has remained the same and CDC is not modifying these assays. The predicate pdmH1 assay consists of two primers and one probe. The modified pdmH1 assay targets the HA gene at the same location and includes minor nucleotide changes to the reverse primer and the probe. The oligonucleotide sequence of the forward pdmH1 primer remains the same.

CDC is removing the H1 assay from the Influenza A Subtyping Kit. The influenza A(H1) viruses targeted by this assay are no longer circulating and thus a diagnostic assay to detect these viruses is unnecessary. Performance of the remaining assays in the Influenza A Subtyping Kit was verified with analytical performance studies.

In addition to evaluating the modified oligonucleotide primers and probes, CDC has evaluated the ZEN™ Double-Quenched Probe technology (manufactured by Integrated DNA Technologies) as an alternate fluorescent hydrolysis probe quencher chemistry. The Influenza A Subtyping Kit assays contain ZEN™ double quenched probes (ZEN probes) that include an internal ZEN™ quencher located nine nucleotides away from the 5' FAM reporter dye in addition to an Iowa Black® FQ quencher (IABkFQ) at the 3' end of the probe.

VII. SUBSTANTIAL EQUIVALENCE COMPARISON

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (K140851) will serve as the predicate for the intended change. See the following table for a detailed comparison.

Device Comparison

	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel, Influenza A Subtyping Kit (K140851)	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel, Influenza A Subtyping Kit (VER 2)
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/H1, A/H3, and/or A/H1pdm09 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/H1 and A/H3 were the predominant influenza A viruses in circulation and during a season when the A/H1pdm09 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 3+ facility is available to receive and culture specimens.	Minor changes to the intended use were made to reflect the current WHO nomenclature of 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 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.	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	Universal influenza A viruses (animal and human), Swine-origin influenza A viruses, Influenza A subtypes: seasonal A(H1), A(H3), A(H1)pdm09	Universal influenza A viruses (animal and human), Swine-origin influenza A viruses, Influenza A subtypes: seasonal A(H3) and A(H1)pdm09
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.	Same
Nucleic Acid Extraction	Yes	Same
Extraction Method	• 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 • Qiagen QIAcube – QIAamp® DSP Viral RNA Mini Kit, Qiagen • NucliSENS® easyMAG®, bioMerieux	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
PCR Technology	Real-Time RT-PCR	Same
Required Instrumentation	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4	Same
Probe Quenching Molecule	Black Hole Quencher Probe® (BHQ-1)	ZEN™ Double-Quenched Probe (InfA, H3, pdmInfA, pdmH1, and RP assays) OR Black Hole Quencher Probe® (InfA, H3, pdmInfA, pdmH1, and RP assays)
Oligonucleotides	H1 Assay-Targets a region of the HA gene H3 assay-Targets a region of the HA gene pdmH1 assay-Targets a region of the HA gene InfA assay-Targets a conserved region of the matrix gene in Influenza A viruses pdmInfA-Targets a conserved region of the nucleoprotein gene in Influenza A(H1N1)pdm09 viruses	Gene targets of the oligonucleotide assays are the same as the predicate; minor changes to the pdmH1 oligonucleotide sequences have been made; the H1 assay is not included in this version of the Influenza A Subtyping Kit

VIII. ANALYTICAL PERFORMANCE EVALUATION

Analytical Sensitivity - Limit of Detection Study (LOD)

Analytical sensitivity of the pdmH1 assay of the Influenza A Subtyping Kit was demonstrated by determining the LOD using Quanta qScript™ and Invitrogen SuperScript™ enzyme kits. Characterized viruses of known 50% infectious dose titers (ID_{50}/mL) were extracted, and the RNA

was serially diluted and tested (n=3 replicates) in order to determine an apparent endpoint range. The LOD for each primer and probe set was confirmed by testing extraction replicates (n=20) of the highest virus dilution where ≥ 95% of all replicates tested positive. Virus dilutions were prepared in virus transport medium containing human A549 cells to emulate clinical specimen matrix. The lowest concentration where the InfA, pdmInfA, and pdmH1 primer and probe sets demonstrated uniform detection was reported as the LOD. The results are summarized in the table below.

pdmH1 Assay LOD Summary

Influenza Virus Tested	Influenza Strain Designation	LOD (ID_{50}/mL)	
		Invitrogen SuperScript™	Quanta qScript™
A(H1)pdm09	A/West Virginia/01/2016	$10^{0.9}$	$10^{0.9}$
	A/California/07/2009	$10^{3.1}$	$10^{3.8}$

A range finding study was also performed for the unmodified H3 assay to demonstrate LOD equivalency between the currently cleared H3 BHQ probe and the H3 ZEN probe. Characterized virus of a known 50% egg infectious dose titer (EID_{50}/mL) was extracted, and the RNA was serially diluted and tested (n=3 replicates) in order to determine an apparent endpoint range.

A/Hong Kong/4801/2014 LOD Equivalency Data for H3 assay

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	H3 IVD BHQ	H3 ZEN	H3 IVD BHQ	H3 ZEN
$10^{4.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{3.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{2.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{1.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{0.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
$10^{-0.1}$	-	-	-	-
$10^{-1.1}$	-	-	-	-

Analytical Sensitivity - Inclusivity Testing

Inclusivity testing was conducted to demonstrate the capability of the oligonucleotide primers and probes in the Influenza A Subtyping Kit to detect strains of influenza A(H1)pdm09 viruses representative of different geographic locations and phylogenetic clades. Inclusivity testing was performed with ten representative H1pdm09 viruses at or near the established LOD. The viruses were grown to high titer, harvested, and serially diluted to near the LOD of the assays. The diluted influenza A(H1)pdm09 viruses were extracted and tested in triplicate with the InfA, pdmInfA, and pdmH1 assays to demonstrate reactivity. Inclusivity of the Influenza A Subtyping Kit was evaluated

with both enzyme systems (i.e., Invitrogen SuperScript™ and Quanta qScript™) and one cleared extraction method. The Influenza A Subtyping Kit was reactive with all H1pdm09 isolates that were tested. The inclusivity results are summarized in the table below.

Inclusivity Results of the Influenza A Subtyping Kit

Influenza Virus Strain Identification	Subtype	ID_{50}/ mL	Invitrogen SuperScript™			Quanta qScript™		
			InfA	pdm InfA	pdm H1	InfA	pdm InfA	pdm H1
A/California/04/2009	A(H1N1)pdm09	$10^{2.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/California/07/2009	A(H1N1)pdm09	$10^{3.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Colorado/14/2012	A(H1N1)pdm09	$10^{1.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Florida/27/2011	A(H1N1)pdm09	$10^{1.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Florida/62/2014	A(H1N1)pdm09	$10^{2.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Maryland/13/2012	A(H1N1)pdm09	$10^{1.0}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Minnesota/03/2011	A(H1N1)pdm09	$10^{3.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/North Carolina/4/2014	A(H1N1)pdm09	$10^{3.3}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Utah/13/2016	A(H1N1)pdm09	$10^{1.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Washington/24/2012	A(H1N1)pdm09	$10^{2.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)

Analytical Specificity – Cross-Reactivity

Cross-reactivity of the Influenza A Subtyping Kit was evaluated by testing influenza A(H1) virus strains representing diverse geographic locations and different sources. Samples were tested in triplicate using RNA extracted from high titer preparations of viruses ($\geq 10^6$ EID_{50}/mL). Cross-reactivity testing of the Influenza A Subtyping Kit was evaluated with both enzyme systems (i.e., Invitrogen SuperScript™ and Quanta qScript™) and one cleared extraction method. The results are shown in the following table.

Influenza Viruses for Cross-Reactivity Testing

Strain Designation	Subtype	ID_{50}/mL	Invitrogen SuperScript™			Quanta qScript™		
			InfA	pdm InfA	pdm H1	InfA	pdm InfA	pdm H1
A/Brisbane/59/07	A(H1N1)	$10^{8.4}$	(+) 3/3	-	-	(+) 3/3	-	-
A/Hawaii/15/2001	A(H1N1)	$10^{8.1}$	(+) 3/3	-	-	(+) 3/3	-	-
A/Iowa/1/2006	A(H1N1v)	$10^{8.2}$	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3
A/Texas/14/2008	A(H1N1v)	$10^{8.3}$	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3
A/Ohio/09/2015	A(H1N1v)	$10^{7.7}$	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3	(+) 3/3
A/Minnesota/19/2011	A(H1N2v)	$10^{7.1}$	(+) 3/3	(+) 3/3	-	(+) 3/3	(+) 3/3	-

IX. CLINICAL PERFORMANCE EVALUATION

The clinical performance of oligonucleotide primer and probe sets of the Influenza A Subtyping Kit were evaluated using retrospective clinical samples collected during the 2011-2012 and 2013-2014 influenza seasons. Clinical samples from the 2015-2016 influenza season that produced aberrant results with the currently cleared pdmH1 assay and were confirmed by genetic sequence analysis were also tested to validate reactivity with the updated assay. A total of forty-two samples that were previously determined to be positive with A(H1)pdm09 influenza virus were evaluated with the InfA, pdmInfA, and pdmH1 assays. Samples that yielded inconclusive results were excluded from the analysis. A total of thirty-two samples that were previously determined to be positive with A(H3) influenza virus were evaluated with the InfA and H3 assays. Clinical testing was performed with both enzymes approved for use with the kit and one of the currently cleared extraction methods. The results are summarized in the tables below.

Retrospective Positive Clinical Study Results-A(H1)pdm09 Comparison

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Positives	% Positive Agreement (95% CI)	# of Positives	% Positive Agreement (95% CI)
BW	1/1	100.0 (20.7-100.0)	1/1	100.0 (20.7-100.0)
NPS, NS	34/35	97.1 (85.5-99.5)	33/33	100.0 (89.6-100.0)
NW	4/4	100.0 (51.0-100.0)	4/4	100.0 (51.0-100.0)
TS	2/2	100.00 (34.2-100.0)	2/2	100.00 (34.2-100.0)

Retrospective Positive Clinical Study Results-A(H3) Comparison

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Positives	% Positive Agreement (95% CI)	# of Positives	% Positive Agreement (95% CI)
NA	1/1	100.0 (20.7-100.0)	1/1	100.0 (20.7-100.0)
NPS, NS	30/30	100.0 (88.7-100.0)	30/30	100.00 (88.7-100.00)
NW	1/1	100.0 (20.7-100.0)	1/1	100.0 (20.7-100.0)

The Influenza A Subtyping Kit oligonucleotide primer and probe sets were also evaluated in specimens that tested negative for influenza A with the CDC Human Influenza rRT-PCR Diagnostic Panel and were obtained from a clinical study conducted during the 2011-2012 influenza season. The specimens that tested negative for influenza A with the currently cleared CDC Human Influenza rRT-PCR Diagnostic Panel were utilized as the comparator for the updated Influenza A Subtyping Kit. For the pdm assays a total of fifty-three samples were tested and for the H3 assay a total of thirty samples were tested. Samples that yielded inconclusive results were excluded from the analysis. The results are summarized in the tables below.

Retrospective Negative Clinical Study Results-A(H1)pdm09 Comparison

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Negatives[1]	% Negative Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NPS	53/53	100.00 (93.2-100.0)	52/52	100.00 (93.1-100.0)

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

Retrospective Negative Clinical Study Results-A(H3) Comparison

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Negatives[1]	% Negative Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NPS	29/29	100.00 (88.3-100.0)	28/28	100.00 (87.9-100.0)

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

X. CONCLUSION

The modification of the CDC Human Influenza Virus rRT-PCR Diagnostic Panel, Influenza A Subtyping Kit to ensure comprehensive detection of influenza A(H1)pdm09 viruses does not substantially change the device. Analytical and clinical data demonstrate that the performance of the device to detect influenza A(H1)pdm09 and influenza A(H3) viruses is accomplished with high positive and negative percent agreement in a manner substantially equivalent to the predicate.