DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Centers For Disease Control And Prevention
Yon Yu, Pharm. D.
Associate Director For Regulatory Affairs
1600 Clifton Road, NE MS-C18
Atlanta, GA 30329-4027

December 1, 2015

Re: K153148
Trade/Device Name: CDC Human Influenza Virus Real- Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit (VER 3)
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: II
Product Code: OZE, NSU, NXD
Dated: October 29, 2015
Received: October 30, 2015

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,

Tamara V. Feldblyum -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)*
K153148

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

Indications for Use *(Describe)*
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/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.

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 a 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.

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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9. 510(k) Summary

I. GENERAL INFORMATION

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Date Prepared: October 22, 2015

II. DEVICE INFORMATION

Proprietary Name:	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit (VER 3)
Common Name:	Influenza A/H5 Subtyping Kit
Regulation Section:	866.3980-Respiratory viral panel multiplex nucleic acid assay
Subsequent Regulation Sections:	862.2570-Instrumentation for clinical multiplex test systems 866.3332-Reagents for detection of specific novel influenza A viruses
Device Classification:	Class II
Product Code:	OZE
Subsequent Product Codes:	NSU, NXD
Panel:	Microbiology

III. PREDICATE DEVICE

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit (K141859)

IV. DEVICE DESCRIPTION

The CDC Human Influenza Real-Time RT-PCR Diagnostic Panel Influenza A/H5 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/H5 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 viruses (InfA) were selected from highly conserved regions of the matrix (M) protein. Oligonucleotide primers and probes for characterization and differentiation of avian influenza A/H5 (Asian lineage) viruses (H5a and H5b) were selected from highly conserved regions of their HA genes. The Influenza A/H5 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/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;
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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.

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 a 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.

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VI. TECHNOLOGICAL CHARACTERISTICS

As circulating Asian lineage highly pathogenic avian influenza (HPAI) viruses continue to evolve, the HA gene target of certain phylogenetic groups has acquired changes in the oligonucleotide primer and probe regions that require modification to ensure comprehensive detection of circulating influenza A/H5 clades. The predicate Influenza A/H5 Subtyping Kit (K141859) includes reagents for two assays, referred to as H5a and H5b, specifically detecting influenza A H5 Asian lineage viruses. The predicate H5a assay consists of two primers and two probes. The predicate H5b assay consists of two primers and one probe. The oligonucleotide primers and probes used in the modified H5a and H5b assays target the HA gene at the same locations and include a total of five primers and three probes for H5a and three primers and one probe for H5b. 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/H5 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/H5 Subtyping Kit (K141859) will serve as the predicate for the intended change. See the table below for a detailed comparison.

Device Comparison

	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel, Influenza A/H5 Subtyping Kit (K141859)	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Diagnostic Panel, Influenza A/H5 Subtyping Kit**
Intended Use	The Influenza A/H5 Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument in conjunction with clinical and epidemiological information: • For the presumptive identification of virus in patients who may be infected with influenza A subtype A/H5 (Asian lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors; • To provide epidemiologic information for surveillance of circulating influenza viruses.	Same

	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. 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 3+ facility is available to receive and culture specimens. All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.	
Organism Detected	Universal influenza A viruses (animal and human) and Influenza A/H5 Subtype (Asian lineage) viruses.	Same
Specimen Types	Human respiratory specimens and viral culture.	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, H5a, H5b, and RP assays) OR Black Hole Quencher Probe® (InfA and RP assays)
Oligonucleotides	H5a assay-Targets a region of the HA gene H5b assay-Targets a region of the HA gene InfA assay-Targets a conserved region of the matrix gene in Influenza A viruses	Gene targets of the oligonucleotide assays are the same as the predicate; minor changes to the oligonucleotide sequences have been made

VIII. ANALYTICAL PERFORMANCE EVALUATION

Analytical Sensitivity - Limit of Detection Study (LOD**)**

Analytical sensitivity of the Influenza A/H5 Subtyping Kit was demonstrated by determining the LOD using Quanta qScript™ and Invitrogen SuperScript™ enzyme kits. Characterized viruses of known 50% infectious dose titers (EID_{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 and both H5a and H5b primer and probe sets demonstrate uniform detection was reported as the LOD. The results are summarized in the table below.

LOD Summary

Influenza Virus Tested	Influenza Strain Designation	LOD (EID_{50}/mL)	
		Invitrogen SuperScript™	Quanta qScript™
A/H5N1	A/Vietnam/1203/2004×A/Puerto Rico/8/34 reassortant (A/Vietnam/1203/2004 PR8- VNH5N1-PR8/CDC-RG)	$10^{3.8}$	$10^{2.4}$
	A/duck/Vietnam/NCVD-1544/2012	$10^{3.1}$	$10^{3.1}$
A/H5N8	A/gyrfalcon/Washington/41088-6/2014	$10^{3.35}$	$10^{3.35}$

Analytical Sensitivity - Inclusivity Testing

Inclusivity testing was conducted to demonstrate the capability of the oligonucleotide primers and probes in the Influenza A/H5 Subtyping Kit to detect strains of influenza A/H5 viruses (Asian lineage) representative of different geographic locations and phylogenetic clades at or near the established LOD. Inclusivity testing was performed with sixteen representative H5 viruses (Asian lineage). A virus of the phylogenetic clade 2.2.2.1 was unavailable for testing, therefore reactivity of the probes with A/Bangladesh/3222/2011 was performed *in silico*. The remaining fifteen viruses were grown to high titer, harvested, and serially diluted to near the LOD of the assays. The diluted influenza A/H5 viruses were extracted and tested in triplicate with the InfA, H5a, and H5b assays to demonstrate

reactivity. Inclusivity of the Influenza A/H5 Subtyping Kit was evaluated with both enzyme systems (i.e. Invitrogen SuperScript™ and Quanta qScript™) and one cleared extraction method.

The Influenza A/H5 Subtyping Kit was reactive with all H5 (Asian lineage) isolates that were tested and predicted to be reactive with the influenza A/Bangladesh/3222/2011 *in silico*. The inclusivity results are summarized in the table below.

Inclusivity Results of the Influenza A/H5 Subtyping Kit

Influenza Virus Strain Identification	Subtype	Clade	EID_{50}/ mL	Invitrogen SuperScript™			Quanta qScript™		
				InfA	H5a	H5b	InfA	H5a	H5b
A/Northern pintail/Washington/40964/2014	H5N2	2.3.4.4	$10^{3.4}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/common magpie/Hong Kong/645/2006	H5N1	2.3.4	$10^{3.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/chicken/Bangladesh/11rs-1984-30/2011	H5N1	2.3.4.2	$10^{3.75}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/chicken/Vietnam/NCVD-279/2009	H5N1	2.3.4.3	$10^{4.25}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Cambodia/W0526301/2012	H5N1	1.1	$10^{3.4}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Cambodia/R040505/2007	H5N1	1.1	$10^{3.5}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/duck/Hunan/795/2002	H5N1	2.1.1	$10^{3.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Indonesia/NIHRD11771/ 2011	H5N1	2.1.3.2a	$10^{4.4}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/turkey/Turkey/1/2005	H5N1	2.2.1	$10^{4.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Egypt/1050/NAMRU3/2013	H5N1	2.2.1	$10^{3.4}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/Bangladesh/3233/2011 (*in silico* analysis)	H5N1	2.2.2.1	--	+	+	+	+	+	+
A/common magpie/Hong Kong/5052/2007	H5N1	2.3.2.1	$10^{4.75}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/chicken/Bangladesh/ 42010/2012	H5N1	2.3.2.1a	$10^{3.2}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/duck/Vietnam/NCVD-672/2011	H5N1	2.3.2.1b	$10^{3.8}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/goose/Guiyang/337/2006	H5N1	4	$10^{4.1}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)
A/chicken/Vietnam/NCVD-1088/2013	H5N1	7.2	$10^{3.9}$	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)	3/3 (+)

Analytical Specificity –Cross-Reactivity

Cross-reactivity of the Influenza A/H5 Subtyping Kit was evaluated by testing influenza A viruses of different types and subtypes that include viruses 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/H5 Subtyping Kit was evaluated with the Invitrogen Superscript™ enzyme system and one cleared extraction method. The results are summarized in the tables below.

Human Influenza Viruses for Cross-Reactivity Testing

Virus Designation	Subtype	EID_{50}/mL	Invitrogen SuperScript™		
			InfA	H5a	H5b
A/Brisbane/59/07	A(H1N1)	$10^{8.4}$	(+) 3/3	-	-
A/Fujian Gulou/1896/2009		$10^{9.1}$	(+) 3/3	-	-
A/Perth/16/2009	A(H3N2)	$10^{8.9}$	(+) 3/3	-	-
A/Texas/50/2012		$10^{9.2}$	(+) 3/3	-	-
A/California/07/09	A(H1N1)pdm09	$10^{8.4}$	(+) 3/3	-	-
A/Washington/24/2012		$10^{8.5}$	(+) 3/3	-	-
B/Brisbane/60/2008	B(Victoria lineage)	$10^{9.3}$	-	-	-
B/Montana/5/2012		$10^{8.4}$	-	-	-
B/Wisconsin/01/2010	B(Yamagata lineage)	$10^{9.2}$	-	-	-
B/Massachusetts/02/2012		$10^{9.2}$	-	-	-

Animal Influenza Viruses for Cross-Reactivity Testing

Species	Virus	Type	EID_{50}/ mL	Invitrogen SuperScript™		
				InfA	H5a	H5b
Swine	A/swine/Wisconsin/125/1997	SW-H1N1	$10^{7.9}$	(+) 3/3	-	-
Swine	A/Maryland/12/1991	SW-H1N1	$10^{9.0}$	(+) 3/3	-	-
Swine	A/Iowa/1/2006	SW-H1N1	$10^{9.0}$	(+) 3/3	-	-
Avian	A/chicken/Pennsylvania/298101 -4/2004	H2N2	$10^{9.5}$	(+) 3/3	-	-
Canine	A/canine/Florida/43/2004	H3N8	$10^{7.2}$	(+) 3/3	-	-
Equine	A/equine/Ohio/01/2003	EQ-H3N8	$10^{7.5}$	(+) 3/3	-	-
Avian	A/chicken/Alabama/1975	H4N8	$10^{10.0}$	(+) 3/3	-	-

Avian	A/duck/Singapore-Q/F119-3/1997	H5N3	$10^{8.5}$	(+) 3/3	(+) 3/3	(+) 3/3
Avian	A/duck/Pennsylvania/1969	H6N1	$10^{9.2}$	(+) 3/3	-	-
Avian	A/chicken/California/32213-1/2000	H6N2	$10^{9.1}$	(+) 3/3	-	-
Avian	A/chicken/New York/13237-6/1998	H6N8	$10^{10.0}$	(+) 3/3	-	-
Avian	A/chicken/New Jersey/15906-9/1996	H11N1	$10^{6.5}$	(+) 3/3	-	-
Avian	A/duck/Memphis/546/1974	H11N9	$10^{9.8}$	(+) 3/3	-	-
Avian	A/Taiwan/2/2013	H6N1	$10^{10.2}$	(+) 3/3	-	-
Avian	A/Anhui/1/2013	H7N9	$10^{10.9}$	(+) 3/3	-	-
Avian	A/mallard/Netherlands/12/2000	H7N3	$10^{9.5}$	(+) 3/3	-	-
Avian	A/chicken/Arkansas/10/2008	H7N3	$10^{9.9}$	(+) 3/3	-	-
Avian	A/Bangladesh/0994/2011	H9N2	$10^{10.5}$	(+) 3/3	-	-
Swine	A/Indiana/21/2012	H3N2v	$10^{9.4}$	(+) 3/3	-	-
Swine	A/Minnesota/11/2010	H3N2v	$10^{9.2}$	(+) 3/3	-	-

Exclusivity Testing

An exclusivity study was performed to demonstrate the specificity of each primer and probe set of the Influenza A/H5 Subtyping Kit when tested with common non-influenza human respiratory viruses, respiratory bacteria, and commensal organisms of the human respiratory tract. All organisms used in the study were propagated, titered, and characterized to confirm identity prior to testing. Nucleic acids were purified from thirty-five (35) non-influenza organisms (16 viruses, 18 bacteria, and 1 yeast) representing common respiratory pathogens or flora commonly present in specimens collected from the human nasopharynx region. High titer preparations of bacteria and yeast, generally greater than or equal to10^6 cfu/mL, and non-influenza respiratory virus preparations at concentrations greater than 10^6 $TCID_{50}$/mL were tested (except in cases where production of high titer virus stock was not possible, e.g. parainfluenza virus type 2). The Influenza A/H5 Subtyping Kit was evaluated with the Invitrogen Superscript™ enzyme system and one cleared extraction method. The results are summarized in the table below.

Exclusivity results with Respiratory Pathogens and Flora

Organism Tested			Invitrogen SuperScript™		
Bacteria and Yeast	Strain	cfu/mL	InfA	H5a	H5b
Bordetella pertussis	A639	$10^{8.3}$	-	-	-
Candida albicans (yeast)	2001-21-196	$10^{8.8}$	-	-	-
Chlamydia pneumoniae	TW183	40 IFU/mL[1]	-	-	-
Corynebacterium diphtheriae	-	10^{10}	-	-	-
Escherichia coli	K12	$10^{9.6}$	-	-	-
Streptococcus pyogenes	7790-06	$10^{7.5}$	-	-	-
Haemophilus influenzae	M15709	$10^{6.4}$	-	-	-
Lactobacillus plantarum	-	$10^{8.8}$	-	-	-
Legionella pneumophila	-	$10^{7.1}$	-	-	-
Moraxella catarrhalis	M15757	$10^{9.5}$	-	-	-
Mycobacterium tuberculosis	H37Rv	100 ng/uL[2]	-	-	-
Mycoplasma pneumoniae	M129	$10^{7.7}$	-	-	-
Neisseria elongata	-	$10^{8.6}$	-	-	-
Neisseria meningitidis	M2578	$10^{7.9}$	-	-	-
Pseudomonas aeruginosa	-	$10^{10.5}$	-	-	-
Staphylococcus aureus	-	$10^{10.7}$	-	-	-
Staphylococcus epidermidis	-	$10^{10.5}$	-	-	-
Streptococcus pneumoniae	249-06	$10^{6.6}$	-	-	-
Streptococcus salivarius	SS1672	$10^{8.4}$	-	-	-
Organism Tested			Invitrogen SuperScript™		
Viruses	Strain	$TCID_{50}$/mL	InfA	H5a	H5b
Human adenovirus type 1	Ad.71	$10^{9.2}$	-	-	-
Human adenovirus type 7a	S-1058	$10^{7.1}$	-	-	-
Human parainfluenza virus type 1	-	3.0 ng/µL[2]	-	-	-
Human parainfluenza virus type 2	Greer	$10^{3.1}$	-	-	-
Human parainfluenza virus type 3	C-243	$10^{7.9}$	-	-	-
Respiratory syncytial virus	CH93-18b	$10^{6.8}$	-	-	-
Human rhinovirus type A	1A	$10^{5.8}$	-	-	-
Enterovirus	Echo 6	$10^{6.9}$	-	-	-
Human coronavirus	299E	31.6ng/µL[2]	-	-	-
Human coronavirus	OC43	50.4ng/µL[2]	-	-	-
Herpes simplex virus	KOS	$5 \times 10^{7.75}$	-	-	-
Varicella-zoster virus	AV92-3:H	$5 \times 10^{3.75}$	-	-	-
Epstein-Barr virus	B95-8	1.7 ng/µL[2]	-	-	-
Measles virus (*Paramyxoviridae*)	Edmonston	$5 \times 10^{4.5}$	-	-	-
Mumps virus	Enders	$5 \times 10^{6.5}$	-	-	-
Human Cytomegalovirus	AD-169	$5 \times 10^{6.25}$	-	-	-

[1]Organism quantified by Infectious Forming Units (IFU)
[2]Organism nucleic acid quantified by spectrophotometry

IX. CLINICAL PERFORMANCE EVALUATION

The clinical performance of oligonucleotide primer and probe sets of the Influenza A/H5 Subtyping Kit were evaluated using contrived samples of grown virus added to an A549 cell suspension to simulate positive clinical samples. A total of fifty positive contrived samples in high, moderate, and low concentrations were evaluated. In addition, sixty-five specimens that tested negative for influenza A with the CDC Human Influenza rRT-PCR Diagnostic Panel that were obtained from a clinical study conducted during the 2011-2012 influenza season were evaluated. Testing was performed with both enzymes cleared for use with the kit. The results are summarized in the table below.

Clinical Performance Evaluation Results

Enzyme Utilized	# of Positives[1]	% Positive Agreement (95% CI)	# of Negatives[2]	% Negative Agreement (95% CI)
Quanta BioSciences qScript™	50/50	100 (92.9 – 100.00)	65/65	100 (94.4 – 100.00)
Invitrogen SuperScript™	44/50	88.0 (76.2 - 94.4)	65/65	100 (94.4 – 100.00)

[1]Proportion of contrived samples correctly identified as positive by both influenza A H5a and H5b primer and probe sets.
[2]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/H5 Subtyping kit to ensure comprehensive detection of circulating influenza A/H5 clades does not substantially change the device. Analytical and clinical data demonstrate that the performance of the device to detect Asian lineage influenza A H5 viruses is accomplished with high positive and negative percent agreement in a manner substantially equivalent to the predicate.

XI. REFERENCES

Lednicky JA, Villanueva JM, Burke SA, Shively R, Shaw MW, Daniels DE, Hamilton SB, Donis RO. 2010. Validation of a method for preparing influenza H5N1 simulated samples. J Virol Methods. 2010 Aug;167(2):125-31.