K132508

510(k) Summary

Submitted by
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Proprietary Name
CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel

Common or Usual Name

Human Influenza Virus Real-time RT-PCR Diagnostic Panel

Regulatory Information

Classification Regulation Section:	866.3332- Reagents for detection of specific novel influenza A viruses
Subsequent Regulation Sections:	866.3980- Respiratory viral panel multiplex nucleic acid assay 862.2570- Instrumentation for clinical multiplex test systems

Classification: Class II
Classification Product Code: OQW
Subsequent Product Codes: NSU, NXD, OEP
Panel: Microbiology

Predicate Device
CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K130551)

Device Description
The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is used in real-time RT-PCR assays (rRT-PCR) on the ABI 7500 Fast Dx Real-Time PCR Instrument. The device consists of oligonucleotide primers and dual-labeled hydrolysis (TaqMan®) probes to

be used in rRT-PCR for the in vitro qualitative detection and characterization of human influenza viruses from viral RNA in respiratory specimens from patients presenting with influenza-like illness (ILI).

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is based on technology which is used in many molecular diagnostic assays. rRT-PCR assays are one-tube assays that first reverse-transcribe specific regions of RNA into cDNA copies. The cDNA then serves as a template for a polymerase chain reaction that utilizes thermocyclic heating and cooling of the reaction to logarithmically amplify a specific region of DNA. The probe anneals to a specific internal target sequence located between the target loci of the forward and reverse primers. During the extension phase of the PCR cycle, the 5' exonuclease activity of Taq polymerase degrades any probe molecules hybridized to amplified target sequence, causing the reporter dye to separate from the quencher dye, and generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle. The amplification of each target is reflected by a logarithmic increase in fluorescence in comparison to the background signal.

Intended Use

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel 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 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;

- 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 NPS, NS, TS, NA, NW, and NPS/TS) and lower respiratory tract specimens (including BAL, BW, TA, sputum and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;

- 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 NPS, NS, TS, NA, NW, and NPS/TS) from human patients with signs and symptoms of respiratory infection and/or from viral culture;

- 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 epidemiological 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 epidemiological 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.

Indications for Use

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel 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 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;

- 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 NPS, NS, TS, NA, NW and NPS/TS) and lower respiratory tract specimens (including BAL, BW, TA, sputum and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;
- 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 NPS, NS, TS, NA, NW, and NPS/TS) from human patients with signs and symptoms of respiratory infection and/or from viral culture.
- 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 epidemiological information for surveillance of circulating influenza viruses.

Technological Characteristics

The changes proposed to the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel subject to this 510(k) modify the device to add the capability to differentiate influenza B viruses, but do not represent a change in the fundamental technology.

Substantial Equivalence Comparison

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K130551) will serve as the predicate for the intended change. See Table 8-1 for a detailed comparison.

Table 8-1: Device Comparison

	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K130551)	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel with Influenza B Lineage Genotyping Assay
Intended Use	The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is intended for use in Real-time RT-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 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs), and lower respiratory tract specimens (including bronchoalveolar lavages, bronchial washes, tracheal aspirates, sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture • For determination of the subtype of seasonal human	The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is intended for use in Real-time RT-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 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs), and lower respiratory tract specimens (including bronchoalveolar lavages, bronchial washes, tracheal aspirates, sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture • 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, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs), and lower respiratory tract specimens (including bronchoalveolar lavages, bronchial washes, tracheal aspirates, sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture • 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 epidemiological information for surveillance of the circulating influenza viruses.	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, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs), and lower respiratory tract specimens (including bronchoalveolar lavages, bronchial washes, tracheal aspirates, sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture • For the determination of the genetic lineage of human influenza B virus as B/Victoria or B/Yamagata lineage from viral RNA in upper respiratory tract clinical specimens (including NPS, NS, TS, NA, NW, and NPS/TS) from human patients with signs and symptoms of respiratory infection and/or from viral culture. • 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 epidemiological information for surveillance of the circulating influenza viruses.
Organism Detected	Universal influenza A viruses (animal and human), Swine-origin influenza A viruses, Influenza B viruses, and Influenza A subtypes: seasonal A/H1, A/H3, A/H1pdm09 , and A/H5	Universal influenza A viruses (animal and human), Swine-origin influenza A viruses, Influenza B viruses, Influenza A subtypes: seasonal A/H1, A/H3, A/H1pdm09, and A/H5, Influenza B/Yamagata and B/Victoria lineages
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 and virus culture.	Same except for influenza B genetic lineage determination: only upper respiratory specimens are claimed.
Nucleic Acid Extraction	Yes	Same
Extraction Method	• QIAamp® Viral RNA Mini Kit, Qiagen Inc. • MagNA Pure Compact -Total Nucleic Acid Kit, Roche Applied Science • MagNA Pure Compact – RNA Isolation Kit, Roche Applied Science • MagNA Pure LC - RNA Isolation Kit II, Roche Applied Science • Qiagen QIAcube with QIAamp® Viral RNA Mini Kit, Qiagen Inc. • 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

The CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel may be used with either Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX (Quanta qScript™)

or Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kit (with or without ROX) (Invitrogen SuperScript™).

Analytical Performance Evaluation

Analytical Sensitivity - Limit of Detection Study (LOD)
Analytical sensitivity of the Influenza B Lineage Genotyping Assay 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 to 5 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 InfB and VIC or InfB and YAM primer and probe sets had uniform detection was reported as the LOD. The results are summarized in Table 8-2.

Table 8-2: LOD Summary

Influenza B Virus Lineage	Virus Designation	LOD (EID_{50}/mL)	
		Invitrogen SuperScript™	Quanta qScript™
B/ Victoria	B/Nevada/03/2011	$10^{2.1}$	$10^{1.4}$
B/Yamagata	B/ Texas/06/2011	$10^{3.5}$	$10^{2.8}$

Analytical Sensitivity - Inclusivity Testing
Inclusivity testing was conducted to demonstrate the capability of the oligonucleotide primers and probes in the Influenza B Lineage Genotyping Assay to detect multiple strains of each influenza B virus lineage at low concentrations. Ten influenza B/Victoria lineage and ten influenza B/Yamagata lineage viruses were grown to high titer and harvested. Each virus was serially diluted to near the assay LOD. The diluted influenza B/Victoria lineage and B/Yamagata lineage viruses were extracted and tested in triplicate with the corresponding primer and probe set to demonstrate reactivity. Inclusivity of the Influenza B Lineage Genotyping Assay was evaluated with both enzyme systems (i.e. Invitrogen SuperScript™ and Quanta qScript™) and one cleared extraction method.

The Influenza B Lineage Genotyping Assay was reactive with all isolates of influenza B/Victoria and B/Yamagata lineage viruses. The inclusivity results are summarized in Tables 8-3 and 8-4.

Table 8-3: Analytical Reactivity of the Influenza B/Victoria Lineage Assay

Virus Designation	Lineage	EID_{50}/mL	Invitrogen SuperScript™		Quanta qScript™	
			InfB	VIC	InfB	VIC
B/Bolivia/1526/2010	B/Vic	$10^{1.4}$	3/3	3/3	3/3	3/3
B/Brisbane/33/2008		$10^{2.4}$	3/3	3/3	3/3	3/3
B/Brisbane/60/2008		$10^{1.5}$	3/3	3/3	3/3	3/3
B/Fujian Gulou/1272/2008		$10^{2.9}$	3/3	3/3	3/3	3/3
B/Georgia/07/2010		$10^{3.2}$	3/3	3/3	3/3	3/3
B/Hong Kong/230/2009		$10^{1.2}$	3/3	3/3	3/3	3/3
B/Hong Kong/259/2010		$10^{3.2}$	3/3	3/3	3/3	3/3
B/New Jersey/1/2012		$10^{1.9}$	3/3	3/3	3/3	3/3
B/Nevada/03/2011		$10^{1.2}$	3/3	3/3	3/3	3/3
B/Texas/26/2008		$10^{3.2}$	3/3	3/3	3/3	3/3

Table 8-4: Analytical Reactivity of the Influenza B/Yamagata Lineage Assay

Virus Designation	Lineage	EID_{50}/mL	Invitrogen SuperScript™		Quanta qScript™	
			InfB	YAM	InfB	YAM
B/Wisconsin/1/2010	B/Yam	$10^{2.2}$	3/3	3/3	2/3	3/3
B/Bangladesh/5972/2007		$10^{1.1}$	3/3	3/3	3/3	3/3
B/Bangladesh/7110/2007		$10^{1.6}$	3/3	3/3	3/3	3/3
B/Chongqingyongchuan/18/2007		$10^{1.3}$	3/3	3/3	3/3	3/3
B/Finland/39/2010		$10^{1.9}$	3/3	3/3	3/3	3/3
B/Brisbane/3/2007		$10^{1.4}$	3/3	3/3	3/3	3/3
B/Hubei-Wujiagang/158/2009		$10^{1.2}$	3/3	3/3	3/3	3/3
B/Pennsylvania/7/2007		$10^{2.2}$	3/3	3/3	3/3	3/3
B/Santiago/4364/2007		$10^{2.2}$	3/3	3/3	3/3	3/3
B/Texas/06/2011		$10^{1.2}$	3/3	3/3	3/3	3/3

Analytical Specificity –Exclusivity

An exclusivity study was performed to demonstrate the specificity of each primer and probe set of the Influenza B Lineage Genotyping Assay when tested with influenza B viruses of the other lineage, with influenza A viruses, and 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 (10) influenza B/Victoria lineage and (10) influenza B/Yamagata lineage viruses, eight (8) influenza A viruses of various subtypes that infect humans, and 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 to 10^6 cfu/mL, were tested with the Influenza B Lineage Genotyping Assay. Similarly, influenza 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 B Lineage Genotyping Assay was evaluated with both enzyme kits (i.e. Invitrogen SuperScript™ and Quanta qScript™) and one cleared extraction method.

The lineage-specific primer and probe sets of the Influenza B Lineage Genotyping Assay were evaluated for cross-reactivity with 10 influenza B viruses of the opposite lineage at high titer. No cross-reactivity was detected when tested in triplicate (n=3) with each isolate of the opposite lineage. The results with each lineage-specific primer and probe set are shown in Tables 8-5 and 8-6.

Table 8-5: Cross-Reactivity of the Influenza B/Yamagata Lineage Assay

Virus Designation	Lineage	EID_{50}/mL	Invitrogen SuperScript™		Quanta qScript™	
			InfB	YAM	InfB	YAM
B/Bolivia/1526/2010	B/Vic	$10^{7.4}$	3/3	0/3	3/3	0/3
B/Brisbane/33/2008		$10^{7.4}$	3/3	0/3	3/3	0/3
B/Brisbane/60/2008		$10^{7.5}$	3/3	0/3	3/3	0/3
B/Fujian Gulou/1272/2008		$10^{7.9}$	3/3	0/3	3/3	0/3
B/Georgia/07/2010		$10^{7.2}$	3/3	0/3	3/3	0/3
B/Hong Kong/230/2009		$10^{7.2}$	3/3	0/3	3/3	0/3
B/Hong Kong/259/2010		$10^{8.2}$	3/3	0/3	3/3	0/3
B/New Jersey/1/2012		$10^{7.9}$	3/3	0/3	3/3	0/3
B/Nevada/03/2011		$10^{7.2}$	3/3	0/3	3/3	0/3
B/Texas/26/2008		$10^{7.2}$	3/3	0/3	3/3	0/3

Table 8-6: Cross-Reactivity of the Influenza B/Victoria Lineage Assay

Virus Designation	Lineage	EID_{50}/mL	Invitrogen SuperScript™		Quanta qScript™	
			InfB	VIC	InfB	VIC
B/Wisconsin/1/2010	B/Yam	$10^{8.2}$	3/3	0/3	3/3	0/3
B/Bangladesh/5972/2007		$10^{6.1}$	3/3	0/3	3/3	0/3
B/Bangladesh/7110/2007		$10^{4.6}$	3/3	0/3	3/3	0/3
B/Chongqingyongchuan/18/2007		$10^{7.3}$	3/3	0/3	3/3	0/3
B/Finland/39/2010		$10^{6.9}$	3/3	0/3	3/3	0/3
B/Brisbane/3/2007		$10^{7.4}$	3/3	0/3	3/3	0/3
B/Hubei-Wujiagang/158/2009		$10^{7.2}$	3/3	0/3	3/3	0/3
B/Pennsylvania/7/2007		$10^{7.2}$	3/3	0/3	3/3	0/3
B/Santiago/4364/2007		$10^{6.2}$	3/3	0/3	3/3	0/3
B/Texas/06/2011		$10^{6.2}$	3/3	0/3	3/3	0/3

The Influenza B Lineage Genotyping Assay was also evaluated for cross-reactivity with various influenza A viruses of subtypes that circulate in humans or from animal origin that infect humans. The results of testing RNA isolated from high titer stocks of each influenza A virus are shown in Table 8-7. Each test was performed using Invitrogen SuperScript™ and Quanta qScript™.

Table 8-7: Cross-Reactivity with Influenza A Viruses Infecting Humans

Species	Virus Designation	Subtype	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™			Quanta qScript™		
				InfB	VIC	YAM	InfB	VIC	YAM
Human	A/Brisbane/59/2007	H1N1	$10^{8.4}$	-	-	-	-	-	-
Human	A/California/07/2009	(H1N1)pdm09	$10^{8.4}$	-	-	-	-	-	-
Human	A/Perth/16/2009	H3N2	$10^{8.2}$	-	-	-	-	-	-
Swine	A/Minnesota/19/2011	H1N2v	$10^{7.1}$	-	-	-	-	-	-
Swine	A/Indiana/10/2011	H3N2v	$10^{10.2}$	-	-	-	-	-	-
Avian	A/chicken/Vietnam/NCVD-016/2008	H5N1	$10^{9.1}$	-	-	-	-	-	-
Avian	A/Egypt/NO3072/2010	H5N1	$10^{9.5}$	-	-	-	-	-	-
Avian	A/Bangladesh/0994/2011	H9N2	$10^{10.5}$	-	-	-	-	-	-

The Influenza B Lineage Genotyping Assay was evaluated for cross-reactivity with various respiratory pathogens and flora. The results are presented in Table 8-8.

Table 8-8: Specificity Results with Respiratory Pathogens and Flora

Organism Tested			Invitrogen SuperScript™			Quanta qScript™		
Bacteria and Yeast	Strain	cfu / mL	Inf B	VIC	YAM	InfB	VIC	YAM
Bordetella pertussis	A639	$10^{8.3}$	-	-	-	-	-	-
Candida albicans	2001-21-196	$10^{8.8}$	-	-	-	-	-	-
Chlamydia pneumoniae[1]	TW183	40 IFU/mL	-	-	-	-	-	-
Corynebacterium diphtheriae	NA	10^{10}	-	-	-	-	-	-
Escherichia coli	K12	$10^{9.6}$	-	-	-	-	-	-
Haemophilus influenzae	M15709	$10^{6.4}$	-	-	-	-	-	-
Lactobacillus plantarum	NA	$10^{8.8}$	-	-	-	-	-	-
Legionella pneumophila	NA	$10^{7.1}$	-	-	-	-	-	-
Moraxella catarrhalis	M15757	$10^{9.5}$	-	-	-	-	-	-
Mycobacterium tuberculosis[2]	H37Rv	95 ng/ µL	-	-	-	-	-	-
Mycoplasma pneumoniae	MI-29	$10^{7.7}$	-	-	-	-	-	-
Neisseria elongata	NA	$10^{8.6}$	-	-	-	-	-	-
Neisseria meningitidis	M2578	$10^{7.9}$	-	-	-	-	-	-
Pseudomonas aeruginosa	NA	$10^{10.5}$	-	-	-	-	-	-
Staphylococcus epidermidis	NA	$10^{10.5}$	-	-	-	-	-	-
Staphylococcus aureus	NA	$10^{10.7}$	-	-	-	-	-	-
Streptococcus pneumoniae	249-06 (Thailand)	$10^{6.6}$	-	-	-	-	-	-
Streptococcus pyogenes	7790-06	$10^{7.5}$	-	-	-	-	-	-
Streptococcus salivarius	SS1672	$10^{8.4}$	-	-	-	-	-	-
Viruses	Strain	$TCID_{50}$/mL	Inf B	VIC	YAM	InfB	VIC	YAM
Enterovirus	Echo 6	$10^{6.9}$	-	-	-	-	-	-
Human Adenovirus, type 1	Ad.71	$10^{9.2}$	-	-	-	-	-	-
Human Adenovirus, type 7a	S-1058	$10^{7.1}$	-	-	-	-	-	-
Human Coronavirus virus[2]	OC43	50.4 ng /µL	-	-	-	-	-	-
Human Coronavirus virus[2]	299E	31.6 ng /µL	-	-	-	-	-	-
Human Rhinovirus A	1A	$10^{5.8}$	-	-	-	-	-	-
Human Parainfluenza 1 virus[2]	NA	3.0 ng/ µL	-	-	-	-	-	-
Human Parainfluenza 2 virus	Greer	$10^{3.1}$	-	-	-	-	-	-
Human Parainfluenza 3 virus	C-243	$10^{7.9}$	-	-	-	-	-	-
Respiratory Syncytial virus	CH93-18b	$10^{6.8}$	-	-	-	-	-	-
Herpes Simplex Virus	KOS	$10^{8.4}$	-	-	-	-	-	-
Varicella-zoster Virus	AV92-3	$10^{4.4}$	-	-	-	-	-	-
Epstein Barr Virus[2]	B95-8	1.7 ng/µL	-	-	-	-	-	-
Measles Virus	Edmonston	$10^{5.2}$	-	-	-	-	-	-
Mumps Virus	Enders	$10^{7.2}$	-	-	-	-	-	-
Cytomegalovirus	AD-169	$10^{6.9}$	-	-	-	-	-	-

[1] Organism quantified by Infectious Forming Units (IFU)
[2] Organism quantified by spectrophotometry (ng/µL)

The analytical specificity studies demonstrated that the Influenza B Lineage Genotyping Assay did not cross-react with any non-specific influenza viruses or any of the non-influenza respiratory pathogens or commensal organisms and demonstrated 100% concordance with the expected results.

Precision/Reproducibility

The reproducibility of the InfB and RP primer and probe sets of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel has been demonstrated in previous submissions (K080570 and K101564), so an internal study was performed to demonstrate the precision of the VIC and YAM primer and probe sets. The study design included the parameters of different operators, days, runs within a day, and samples of different concentrations (i.e. low, medium, high negative) as recommended in the FDA Guidance Document "Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection and Differentiation of Influenza Viruses." Determinations of precision estimates were performed using guidelines provided in the Clinical Laboratory Standards Institute document EP5-A2 (Tholen et al.). Each primer and probe set was tested with one influenza strain for which it was designed and using one extraction method and one enzyme kit option. Three samples (moderate, low, and high negative) of each lineage were tested by two operators who individually performed two separate runs per testing day. Testing was performed over 12 days (not necessarily consecutive), however, both operators performed testing on the same day. This design generated 96 results per panel member.

The results of the within-laboratory precision study are presented in Table 8-9.

Table 8-9: Precision Summary

Panel Sample	Primer/ Probe Set	Analyst 1			Analyst 2			Agreement Total	95%CI
		Agreement	Ave. Ct	%CV	Agreement	Ave. Ct	%CV		
B/Victoria Moderate	InfB	48/48	23.86	4.3	48/48	23.96	5.0	96/96	100.0 (96.2 – 100.0)
	VIC	48/48	26.36	3.7	48/48	25.98	5.9	96/96	100.0 (96.2 – 100.0)
B/Victoria Low	InfB	46/48	33.28	4.7	45/48	33.68	6.0	91/96	94.8 (88.4 – 97.8)
	VIC	31/48	37.32	4.5	38/48	36.60	5.0	69/96	71.9 (62.2 – 79.9)
B/Victoria High Negative	InfB	47/48	N/A	N/A	44/48	N/A	N/A	91/96	94.8 (88.4 – 97.8)
	VIC	48/48	N/A	N/A	48/48	N/A	N/A	96/96	100.0 (96.2 – 100.0)
B/Yamagata Moderate	InfB	48/48	26.01	4.0	48/48	25.70	4.4	96/96	100.0 (96.2 – 100.0)
	YAM	48/48	26.70	4.2	48/48	26.14	4.5	96/96	100.0 (96.2 – 100.0)
B/Yamagata Low	InfB	46/48	32.14	3.6	48/48	32.46	3.2	94/96	97.9 (92.7 – 99.4)
	YAM	46/48	32.72	4.0	48/48	32.21	3.9	94/96	97.9 (92.7 – 99.4)
B/Yamagata High Negative	InfB	48/48	N/A	N/A	48/48	N/A	N/A	96/96	100.0 (96.2 – 100.0)
	YAM	47/48	N/A	N/A	48/48	N/A	N/A	95/96	99.0 (94.3 – 99.8)

N/A=not applicable

The within laboratory precision study demonstrated a high concordance with the expected results for each type of sample and a coefficient of variation below 6% or lower. A lower degree of agreement was observed with the VIC primer and probe set with the sample that

was at or near the LOD. Testing of this sample resulted in an average Ct of approximately 37 which is very close to the established cutoff of the assay.

Clinical Performance Evaluation

A clinical study was conducted during the 2011-2012 influenza season to evaluate the performance of the CDC Human Influenza rRT-PCR Diagnostic Panel- Influenza B Lineage Genotyping Assay. Residual material from a total of 1,002 respiratory specimens from patients who were symptomatic for influenza-like illness (ILI) was collected and tested at 6 clinical sites. The clinical study was conducted in accordance with the clinical protocol entitled "Clinical Validation Protocol for Evaluating the CDC Influenza B Lineage Genotyping Assay" [FDA pre-IDE I120636] (Attachment A), following the training of each clinical site. Specimens were excluded from the clinical data analysis for reasons of unknown specimen type (4), lower respiratory specimen (5), inconclusive results (1), or technician error (131; excluded from Quanta qScript™ runs only). After exclusion of specimens there were a total of 992 specimens tested using Invitrogen SuperScript™ and 861 specimens tested using Quanta qScript™.

The study population and specimen types are summarized by age range and specimen type in Table 8-10.

Table 8-10: Study Population and Specimen Type Summary

	Number of Clinical Specimens by Type							
Age Range	NA	NW	NS	NPS	NPS/TS	TS	LR	Unknown
0-16	10	15	51	337	14	2	3	2
17-54	2	6	37	263	14	2	2	2
≥ 55	1	2	25	174	9	4	0	0
Unknown	0	1	0	24	0	0	0	0
Totals	13	24	113	798	37	8	5	4

NA=nasal aspirate, NW=nasal wash, NS=nasal swab, NPS=nasopharyngeal swab, NPS/TS=dual nasopharyngeal and throat swab, TS=throat swab, LR=lower respiratory specimens including bronchoalveolar lavage, bronchial wash, tracheal aspirate, sputum, or lung tissue.

Expected Values

During February 25, 2012, to May 19, 2012, World Health Organization and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories in the United States tested 47,281 respiratory specimens for influenza viruses. Of these, 9,415 (19.9%) were positive: 85% of the positive specimens were positive for influenza A viruses and 15% were positive for influenza B viruses. Among the 5,071 influenza A viruses for which subtyping was performed, 3,680 (72.6%) were influenza A/H3 viruses and 1,391 (27.4%) were 2009 H1N1 influenza viruses.

Prospective Study

The performance of the Influenza B Lineage Genotyping Assay was demonstrated at the clinical sites using both the Invitrogen Superscript™ and Quanta qScript™ enzyme kits. Specimens collected in the study were tested with the InfB and RP primer and probe set from the FDA-cleared CDC Human Influenza Real-Time RT-PCR Diagnostic Panel to establish

the presence of influenza B viral RNA in upper respiratory specimens and confirm adequate specimen collection. In order to determine the performance of the Influenza B Lineage Genotyping Assay, bi-directional nucleic acid sequence analysis of a region of the influenza B hemagglutinin (HA) gene was performed on each specimen containing influenza B viral RNA.

Contemporary influenza B viruses can be phylogenetically and antigenically separated into two major lineages on the basis of the influenza virus HA gene. The divergence of the two antigenically distinct lineages was first recognized during seasonal surveillance during the late 1980s and is represented by the reference viruses, influenza B/Yamagata/16/88 and B/Victoria/2/87 (Rota et al. 1990). An alignment of the full length HA sequences from many viruses from both lineages was used to locate a variable region flanked by two conserved regions that could be used to discriminate viruses from the two different lineages. This variable region was selected intentionally as separate and distinct from the region targeted in the influenza B lineage genotyping assays. Minor genetic variations are found among clinical isolates from each of the two lineages due to the accumulation of mutations within the variable region as the viruses evolve, however, the lineage of these viruses can still be determined by a high percent identity of their nucleic acid sequences to that of representative reference viruses.

A 221-bp fragment containing the selected variable region of the HA gene was amplified by RT-PCR from RNA isolated from each clinical specimen. Each strand of the DNA amplicon was sequenced to obtain 4-fold coverage of an 82-bp region with Phred20 quality sequence data (99% accuracy). Sequence data of each sample were aligned to B/Victoria and B/Yamagata reference virus sequences of the HA gene and the percent homology calculated. A homology exceeding 95% to one lineage reference sequence while showing less than 95% to the other lineage reference sequence confirmed the identity of the influenza B virus. Multiple reference sequences for the B/Yamagata lineage were needed to resolve some samples since there appeared to be persistence of some older influenza B viruses in circulation in the population sampled. The prospective study results of the Influenza B Lineage Genotyping Assay used with either Invitrogen SuperScript™ or Quanta qScript™ enzyme kits are summarized in Tables 8-11 through 8-14 showing the percent agreement and the two-sided 95% confidence interval calculated according to the score method (Garret et al.).

Table 8-11: VIC Comparison using Invitrogen SuperScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NA, NW	2/2	100.0 (34.2 – 100.0)	37/37	100.0 (90.6 – 100.0)
NPS, NS	45/45	100.0 (92.1 – 100.0)	862/863	99.9 (99.3 – 100.0)
NPS/TS	12/12	100.0 (75.8 – 100.0)	25/25	100.0 (86.7 – 100.0)
TS	NA[2]	NA	8/8	100.0 (67.6 – 100.0)

[1]Proportion of true positives or true negatives correctly identified versus the comparator
[2]Not applicable; no positive samples of this specimen type were obtained

Table 8-12: YAM Comparison using Invitrogen SuperScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NA, NW	NA[2]	NA	39/39	100.0 (91.0 – 100.0)
NPS, NS	19/19	100.0 (83.1 – 100.0)	889/889	100.0 (99.6 – 100.0)
NPS/TS	2/2	100.0 (34.2 – 100.0)	35/35	100.0 (90.1 – 100.0)
TS	NA	NA	8/8	100.0 (67.6 – 100.0)

[1]Proportion of true positives or true negatives correctly identified versus the comparator
[2]Not applicable; no positive samples of this specimen type were obtained

Table 8-13: VIC Comparison using Quanta qScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NA, NW	2/2	100.0 (34.2 – 100.0)	22/22	100.0 (85.13 – 100.0)
NPS, NS	44/44	100.0 (92.0 – 100.0)	749/752	99.6 (98.8 – 99.9)
NPS/TS	11/12	91.7 (64.6 – 98.5)	21/21	100.0 (84.5 – 100.0)
TS	NA[2]	NA	8/8	100.0 (67.6 – 100.0)

[1]Proportion of true positives or true negatives correctly identified versus the comparator
[2]Not applicable; no positive samples of this specimen type were obtained

Table 8-14: YAM Comparison using Quanta qScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NA, NW	NA[2]	NA	24/24	100.0 (86.2 – 100.0)
NPS, NS	19/19	100.0 (83.2 – 100.0)	775/777	99.7 (99.1 – 99.9)
NPS/TS	2/2	100.0 (34.2 – 100.0)	31/31	100.0 (89.0 – 100.0)
TS	NA[2]	NA	8/8	100.0 (67.6 – 100.0)

[1]Proportion of true positives or true negatives correctly identified versus the comparator
[2]Not applicable; no positive samples of this specimen type were obtained

Retrospective Study

The prevalence of influenza B viruses within the prospective clinical study population was approximately 8% with the B/Victoria lineage outnumbering B/Yamagata lineage by a factor of 3 to 1. To augment the number of B/Yamagata lineage viruses in the clinical study, additional study specimens were selected from routine influenza surveillance samples collected between September 2012 and January 2013 that had been initially screened for influenza A and B with the FDA-cleared CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Fifty-one specimens identified as influenza B positive were selected for the study and tested using the approved clinical protocol. The retrospective study results of the Influenza B Lineage Genotyping Assay used with either Invitrogen SuperScript™ or Quanta qScript™ enzyme kits are summarized in Table 8-15 through 8-18 showing the percent agreement and the two-sided 95% confidence interval.

Table 8-15: VIC Comparison using Invitrogen SuperScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)
NA, NW	1/1	100.0 (20.7 – 100.0)
NPS, NS	14/14	100.0 (78.5 – 100.0)

[1]Proportion of true positives correctly identified versus the comparator

Table 8-16: YAM Comparison using Invitrogen SuperScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)
NA, NW	3/3	100.0 (43.9 – 100.0)
NPS, NS	29/29	100.0 (88.3 – 100.0)

[1]Proportion of true positives correctly identified versus the comparator

Table 8-17: VIC Comparison using Quanta qScript™

Specimen Type	# of Positives1	% Positive Agreement (95% CI)
NA, NW	1/1	100.0 (20.7 – 100.0)
NPS, NS	14/14	100.0 (78.5 – 100.0)

[1]Proportion of true positives correctly identified versus the comparator

Table 8-18: YAM Comparison using Quanta qScript™

Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)
NA, NW	3/3	100.0 (43.9 – 100.0)
NPS, NS	28/29	96.6 (82.8 – 99.4)

[1]Proportion of true positives correctly identified versus the comparator

Conclusion

The modification of the CDC Human Influenza Virus rRT-PCR Diagnostic Panel to add the capability to differentiate the two major lineages of influenza B viruses does not substantially change the device. Analytical and clinical data demonstrate that the performance of the device to determine the lineage of influenza B viruses is accomplished with high positive and negative percent agreement with the comparator method in a manner substantially equivalent to the predicate.

References

FDA Guidance Document: Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection and Differentiation of Influenza Viruses [Issued July 15, 2011]. Food and Drug Administration, Center for Devices and Radiological Health, Office of *In Vitro* Diagnostic Device Evaluation and Safety

Garrett, P.E., Lasky, F.D., Meier, K.L. 2008. Clinical and Laboratory Standards Institute (CLSI). User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition. CLSI document EP12-A2.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

JAN 14 2014

Centers for Disease Control and Prevention
C/O CAPT. Hye-Joo Kim, Pharm D.,
Associate Director for Regulatory Affairs, NCEZID
1600 Clifton Road, MS-C18,
Atlanta, GA 30333

Re: K132508
Trade/Device Name: CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OZE, OEP, OQW, NXD, NSU
Dated: August 9, 2013
Received: August 12, 2013

Dear Dr. Kim:

This letter corrects our substantially equivalent letter of September 23, 2013.

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 regulation (21 CFR Parts 801 and 809), please contact the Division of Small Manufacturers, International and Consumer Assistance 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" (21CFR 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 Small Manufacturers, International and Consumer Assistance 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

Sally A. Hojvat, M.Sc., Ph.D.
Director,
Division of Microbiology Devices
Office of *In Vitro* Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): K132508

Device Name: **CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel**

Indications for Use:

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel 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 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;
- 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 NPS, NS, TS, NA, NW and NPS/TS) and lower respiratory tract specimens (including BAL, BW, TA, sputum and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;
- 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 NPS, NS, TS, NA, NW, and NPS/TS) from human patients with signs and symptoms of respiratory infection and/or from viral culture;
- 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 epidemiological 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 epidemiological 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.

Prescription Use _X_____ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of Center for Devices and Radiological Health (CDRH)

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