SPECIAL 510(K): DEVICE MODIFICATION
DECISION SUMMARY

510(k) Number: _K181736__

This 510(k) submission contains information/data on modifications made to the applicant's own class II device requiring 510(k). The following items are present and acceptable:

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1. The name and 510(k) number of the applicant's previously cleared device.

510(k) Number	Device Name	Clearance Date	Primary Reason for 510(k) Submission
K172091	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit	8/9/2017	A bundeled 510(k) submission for device modification to add three additional options of nucleic acids extraction instrument and extraction method combinations (QIAGEN EZ1 DSP Virus Kit on the QIAGEN EZ1 Advanced XL instrument, QIAGEN EZ1 RNA Tissue Mini Kit on the QIAGEN EZ1 Advanced XL instrument, and Roche DNA and Viral NA Small Volume Kit on the Roche MagNA Pure 96 instrument) that are acceptable for use with the four previsouly FDA-cleared diagnostic kits of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, the Influenza A/B Typing Kit, the Influenza A Subtyping Kit (Ver 2), the Influenza B Lineage Genotyping Kit, and the Influenza A/H5 Subtyping Kit (Ver 3).

2. Applicant's statement that the **INDICATION/INTENDED USE** of the modified device as described in its labeling **HAS NOT CHANGED** along with the proposed instructions for use.

3. A description of the device **MODIFICATION(S)** in sufficient detail to demonstrate that the **FUNDAMENTAL SCIENTIFIC TECHNOLOGY** of the modified device **has not changed**.

    1) To address recent evolutionary changes in circulating influenza B viruses that may impact the reactivity of the current Influenza B Lineage Genotyping Kit of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel (i.e., the prevalence of specific genetic mutations in the targeted influenza B HA gene is increasing), the CDC Influenza Division modified the existing FDA-cleared device using the following two design approaches for the VIC and YAM assays. The first design approach updated individual bases in the existing primer or probe sequences to eliminate mismatches with the targeted RNA sequences of emerging viruses within each lineage, B/Yamagata or B/Victoria. The targeted locations of the primer and probe design as well as all reaction conditions remain essentially the same as the previously FDA-cleared VIC and YAM assays. These updated assays (primers/probe sets) are named VIC (VER 1.1) and YAM (VER 1.1). The second design approach targeted a different location on the HA gene of each influenza B lineage. This approach used a "standard" probe design typical of the other CDC assays in CDC Human Influenza Real-Time RT-PCR Diagnostic Panel that target a longer genetic region and do not incorporate modified bases or minor-groove-binding molecules to increase annealing temperatures. These modified assays (primers/probe sets) are named VIC (VER 2) and YAM (VER 2).

    2) To incorporate an additional quencher chemistry, ZEN™ (manufactured by Integrated DNA Technologies), to the previously FDA-cleared influenza B (InfB) assay probe as a

manufacturing option. This represents a continuation of previous efforts and submissions by CDC Influenza Division in order to extend this manufacturing option to all assays within the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. The implementation of the alternate oligonucleotide probe quencher chemistry, ZEN™, has been addressed in previous CDC submissions for the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. (e.g., K153148 and K161556).

3) The product instructions for use (package insert) was updated primarily to reflect the redesigned and the modified assays primers and probes. Two different versions of the package insert are available, each version contains one specific primers/probe set for each of the VIC and YAM assays only (i.e., VER 1.1 or VER 2, not both).

4. **Comparison Information** (similarities and differences) to applicant's legally marketed predicate device.

Item	Cleared Device	Modified Device
Features	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2) (K181736)**

Item	Cleared Device	Modified Device
Features	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2) (K181736)**
Intended Use	The Influenza B Lineage Genotyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument in conjunction with clinical and epidemiological information: • For the determination of the genetic lineage of human influenza B viruses as B/Victoria or B/Yamagata lineage from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) from human patients with signs and symptoms of respiratory infection and/or from viral culture; • To provide epidemiologic information for surveillance of circulating influenza viruses. Performance characteristics for influenza B lineage genotyping were established during a season when influenza B/Victoria and B/Yamagata lineages were found in approximately equal proportion. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.	Same
Organisms Detected	Influenza B virus lineages B/Victoria and B/Yamagata	Same
Specimen Types	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs from human patients with signs and symptoms of respiratory infection and/or from viral culture	Same
Technological Characteristics	Real-Time RT-PCR based assays	Same

Item	Cleared Device	Modified Device
Features	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (K172091)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2) (K181736)**
Nucleic Acid Extraction	• QIAamp® DSP Viral RNA Mini Kit, QIAGEN • MagNA Pure Compact –Nucleic Acid Isolation Kit I, Roche • MagNA Pure Compact – RNA Isolation Kit, Roche • MagNA Pure LC – Total Nucleic Acid Kit, Roche • QIAcube – QIAamp® DSP Viral RNA Mini Kit, QIAGEN • NucliSENS® easyMAG®, bioMerieux • EZ1 Advanced XL – EZ1 DSP Virus Kit and EZ1 RNA Tissue Mini Kit, QIAGEN • MagNA Pure 96 - DNA and Viral NA Small Volume Kit, Roche	Same
Master Mix Enzyme	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
Required RT-PCR Instrumentation	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4	Same
RT-PCR Assays	InfB, VIC, and YAM assays	InfB, VIC (VER 1.1), and YAM (VER 1.1) assays InfB, VIC (VER 2), and YAM (VER 2) assays
Probe Quenching	Black Hole Quencher Probe® (BHQ-1) (InfB assay) Black Hole Quencher Plus (BHQ*Plus*) (VIC, YAM assays)	ZEN™ or BHQ-1 (InfB assay) BHQ*Plus* [VIC (VER 1.1) and YAM (VER 1.1) assays] BHQ-1 [VIC (VER 2) and YAM (VER 2) assays]

5. A **Design Control Activities Summary** which includes:

   a) Identification of Risk Analysis method(s) used to assess the impact of the modification on the device and its components, and the results of the analysis.

      The Risk Assessment process used was based on an internal Risk Management Procedure, the CDC Influenza Division's Quality Management System DC -10: FluIVD03 Risk Analysis, which is consistent with the requirements in ISO 14971. Using this procedure, the primary failures/risks associated with modifying and redesigning the VIC and YAM assay primers and probes, as well as introducing the alternate oligonucleotide probe quencher chemistry, ZEN™ (manufactured by Integrated DNA Technologies), to the InfB assay probe, were analyzed.

   b) Based on the Risk Analysis, an identification of the verification and/or validation activities required, including methods or tests used and acceptance criteria to be applied.

      Based on the Risk Analysis, CDC followed its established internal procedures when developing, validating, and implementing the modified and redesigned Influenza B Lineage Genotyping assays. CDC conducted analytical and clinical studies (see Section 6 and Section 7 below) using the modified and redesigned assays to demonstrate that the performance of the modified Influenza B Lineage Genotyping Kit succefully mitigates the identified potential risks to an acceptable level.

6. **Analytical Performance**

   Analytical Sensitivity - Influenza B (InfB) Assay

   A range finding analytical study was performed to demonstrate Limit of Detection (LoD) equivalency between the previously FDA-cleared InfB assay probe quenched with BHQ-1 and the same probe quenched with ZEN. Two characterized influenza vaccine reference viruses of a known 50% egg infectious dose titer (EID_{50}/mL) were extracted using the Roche MagNA Pure Compact RNA Isolation Kit. The RNA was serially diluted and tested (n=3 replicates) in order to determine the apparent endpoint range using both enzymes cleared for use with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. The acceptance criterion for LoD equivalency was defined as demonstrating 100% positivity (3 out of 3 replicates) at either the same endpoint LoD concentration or within a 5-fold dilution of each other. All assays demonstrated similar analytical reactivity in this study (See Table 1 and Table 2 below).

   Table 1: Influenza B (InfB) Assay LoD Equivalency - B/Nevada/03/2011 (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza B/Nevada/03/2011 Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfB (BHQ-1)	InfB (ZEN)	InfB (BHQ-1)	InfB (ZEN)
$10^{4.2}$	3/3	3/3	3/3	3/3

$10^{3.5}$	3/3	3/3	3/3	3/3
$10^{2.8}$	3/3	3/3	3/3	3/3
$10^{2.1}$	1/3	1/3	0/3	0/3
$10^{1.4}$	2/3	0/3	0/3	0/3
$10^{0.7}$	0/3	0/3	0/3	0/3
$10^{0.0}$	0/3	0/3	0/3	0/3

Table 2: Influenza B (InfB) Assay LoD Equivalency - B/Wisconsin/1/2010 (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza B/Wisconsin/1/2010 Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfB (BHQ-1)	InfB (ZEN)	InfB (BHQ-1)	InfB (ZEN)
$10^{4.2}$	3/3	3/3	3/3	3/3
$10^{3.5}$	3/3	3/3	3/3	3/3
$10^{2.8}$	1/3	0/3	0/3	0/3
$10^{2.1}$	0/3	0/3	0/3	0/3
$10^{1.4}$	0/3	0/3	0/3	0/3
$10^{0.7}$	0/3	0/3	0/3	0/3
$10^{0.0}$	0/3	0/3	0/3	0/3

Analytical Sensitivity – Modified and Newly Designed Influenza B Lineage Genotyping Assays

Range finding analytical studies were performed with the previsouly FDA-cleared VIC and YAM assays of the Influenza B Lineage Genotyping Kit, and with the modified VIC and YAM assays (VER 1.1), as well as newly designed VIC and YAM assays (VER 2). These studies were performed to demonstrate the LoD equivalency or the improved analytical reactivity of the VER 1.1 and VER 2 VIC and YAM assays over the previously FDA-cleared VIC and YAM assays with one historical benchmark strain and one current strain of each of the two influenza B virus lineages, B/Victoria and B/Yamagata lineages. The test samples consisted of serially diluted RNA extracted with the Roche MagNA Pure Compact RNA Isolation Kit from characterized virus stocks of

known 50% egg infectious dose titer (EID_{50}/mL). Three replicates per dilution were tested to determine the apparent endpoint range using both enzymes cleared for use with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. The acceptance criterion for LoD equivalency was defined as demonstrating 100% positivity (3 out of 3 replicates) at either the same endpoint LoD concentration or within a 5-fold dilution of each other. While all assays demonstrated similar reactivity with apparent LoD endpoints within one 5-fold dilution of each other for most of the strains tested, the VER 1.1 and VER 2 VIC and YAM assays appeared to show improved analytical reactivity over the previously FDA-cleared VIC and YAM assays for at least some of the strains tested (See Table 3 to Table 6 below).

Table 3: B/Victory (VIC) Assay LoD Equivalency – VER 1.1 and VER 2 VIC Assays - B/Nevada/03/2011 (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza B/Nevada/03/2011 Titer (EID_{50}/mL)	Invitrogen Superscript™				Quanta qScript™			
	InfB	VIC	VIC (VER 1.1)	VIC (VER 2)	InfB	VIC	VIC (VER 1.1)	VIC (VER 2)
$10^{4.2}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.5}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{2.8}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{2.1}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{1.4}$	3/3	3/3	3/3	3/3	3/3	0/3	3/3	3/3
$10^{0.7}$	1/3	0/3	2/3	3/3	0/3	0/3	1/3	0/3
$10^{0.0}$	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
$10^{-0.7}$	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Table 4: B/Victory (VIC) Assay LoD Equivalency - VER 1.1 and VER 2 VIC Assays - B/Maryland/15/2016 (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza B/Maryland/15/2016 Titer (EID_{50}/mL)	Invitrogen Superscript™				Quanta qScript™			
	InfB	VIC	VIC (VER 1.1)	VIC (VER 2)	InfB	VIC	VIC (VER 1.1)	VIC (VER 2)
$10^{4.5}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.8}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

$10^{3.1}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{2.4}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{1.7}$	3/3	3/3	3/3	3/3	3/3	2/3	3/3	3/3
$10^{1.0}$	3/3	0/3	3/3	3/3	0/3	0/3	0/3	0/3
$10^{0.3}$	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
$10^{-0.4}$	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Table 5: B/Yamagata (YAM) Assay LoD Equivalency - VER 1.1 and VER 2 YAM Assays - B/Texas/06/2011 (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza B/Texas/06/2011 Titer (EID_{50}/mL)	Invitrogen Superscript™				Quanta qScript™			
	InfB	YAM	YAM (VER 1.1)	YAM (VER 2)	InfB	YAM	YAM (VER 1.1)	YAM (VER 2)
$10^{4.9}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{4.2}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.5}$	3/3	3/3	3/3	3/3	3/3	3/3	2/3	3/3
$10^{2.8}$	3/3	3/3	0/3	3/3	3/3	2/3	0/3	3/3
$10^{2.1}$	2/3	1/3	1/3	1/3	0/3	2/3	0/3	2/3
$10^{1.4}$	2/3	0/3	0/3	1/3	0/3	1/3	0/3	2/3
$10^{0.7}$	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
$10^{0.0}$	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Table 6: B/Yamagata (YAM) Assay LoD Equivalency - VER 1.1 and VER 2 YAM Assays - B/Texas/81/2016 (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza B/Texas/81/2016 Titer (EID_{50}/mL)	Invitrogen Superscript™				Quanta qScript™			
	InfB	YAM	YAM (VER 1.1)	YAM (VER 2)	InfB	YAM	YAM (VER 1.1)	YAM (VER 2)

$10^{4.3}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.6}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{2.9}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{2.2}$	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
$10^{1.5}$	3/3	2/3	3/3	3/3	3/3	3/3	1/3	2/3
$10^{0.8}$	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
$10^{0.1}$	1/3	0/3	0/3	0/3	0/3	2/3	0/3	0/3
$10^{-0.6}$	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

A confirmation of the LoD for each modified or newly designed assay was performed using a current strain of the corresponding influenza B/Victoria or B/Yamagata lineage. The VER 1.1 and VER 2 VIC and YAM primer and probe sets were tested alongside the currently FDA-cleared InfB assay as required for interpretation by the routine testing algorithm. The confirmatory LoD testing for each primer and probe set was performed on extraction replicates (n=20) of each dilution. The lowest virus concentration where InfB and VIC or InfB and YAM primers and probes sets demonstrated ≥ 95% detection is reported as the confirmed LoD for each virus for the Primers/probe set (See Table 7 below).

Table 7: LoD Confirmation – VER 1.1 and VER 2 VIC and YAM Assays

Influenza B Lineage	Influenza Strain	Assay Design	LoD (EID_{50}/mL)	
			Invitrogen SuperScript™	Quanta qScript™
Victoria	B/Maryland/15/2016	VIC (VER 1.1)	$10^{1.7}$	$10^{1.7}$
		VIC (VER 2)	$10^{1.7}$	$10^{1.7}$
Yamagata	B/Texas/81/2016	YAM (VER 1.1)	$10^{2.2}$	$10^{1.5}$
		YAM (VER 2)	$10^{2.2}$	$10^{1.5}$

Analytical Inclusivity - Modified and Newly Designed Influenza B Lineage Genotyping Assays

Analytical inclusivity testing was performed to demonstrate the capability of the modified VER 1.1 VIC and YAM primers/probe sets and the newly designed VER 2 VIC and YAM primers/probe sets to detect strains of the corresponding influenza B lineage at or near the confirmed LoD. Ten influenza B viruses of each lineage and representative of different geographic locations and phylogenetic clades were selected for this testing. Characterized stocks were serially diluted to concentrations near the LoD of the assays and extracted using the Roche MagNA Pure Compact RNA Isolation Kit. Samples were tested in triplicate with the VER 1.1 and VER 2 VIC and YAM assays with both enzyme systems FDA-cleared for use with the CDC Human Influenza Real-Time

RT-PCR Diagnostic Panel. The analytical inclusivity testing results are summarized in Table 8 and Table 9 below.

Table 8: Analytical Reactivity Testing – VER 1.1 and VER 2 VIC Assays (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza Virus B/Victory Strain Designation	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™		Quanta qScript™	
		VIC (VER 1.1)	VIC (VER 2)	VIC (VER 1.1)	VIC (VER 2)
B/Hong Kong/259/2010	$10^{1.2}$	3/3	3/3	3/3	3/3
B/Bolivia/1526/2010	$10^{2.4}$	3/3	3/3	3/3	3/3
B/Laos/89/2011	$10^{1.2}$	3/3	3/3	3/3	3/3
B/Michigan/09/2011	$10^{1.5}$	3/3	3/3	3/3	3/3
B/New Jersey/01/2012	$10^{1.9}$	3/3	3/3	3/3	3/3
B/Montana/05/2012	$10^{2.4}$	3/3	3/3	3/3	3/3
B/Texas/02/2013	$10^{2.1}$	3/3	3/3	3/3	3/3
B/Florida/103/2016	$10^{0.3}$	3/3	3/3	3/3	3/3
B/Florida/76/2016	$10^{1.5}$	3/3	3/3	3/3	3/3
B/Hong Kong/269/2017	$10^{-0.8}$	3/3	3/3	3/3	3/3

Table 9: Analytical Reactivity Testing - VER 1.1 and VER 2 YAM Assays (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza Virus B/Yamagata Strain Designation	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™		Quanta qScript™	
		YAM (VER 1.1)	YAM (VER 2)	YAM (VER 1.1)	YAM (VER 2)
B/Brisbane/03/2007	$10^{2.4}$	3/3	3/3	3/3	3/3
B/Pennsylvania/07/2007	$10^{2.2}$	3/3	3/3	3/3	3/3
B/Hubei-Wujiagang/158/2009	$10^{2.2}$	3/3	3/3	3/3	3/3
B/Wisconsin/01/2010	$10^{2.9}$	3/3	3/3	3/3	3/3
B/Finland/39/2010	$10^{2.1}$	3/3	3/3	3/3	3/3
B/Estonia/55669/2011	$10^{2.8}$	3/3	3/3	3/3	3/3

B/Taiwan/1242/2011	$10^{3.9}$	3/3	3/3	3/3	3/3
B/Massachusetts/02/2012	$10^{2.2}$	3/3	3/3	3/3	3/3
B/Phuket/3073/2013	$10^{2.9}$	3/3	3/3	3/3	3/3
B/Guangdong-Liwan/1133/2014	$10^{3.9}$	3/3	3/3	3/3	3/3

Analytical Exclusivity - Modified and Newly Designed Influenza B Lineage Genotyping Assays

Potential cross-reactivity of the modified VER 1.1 VIC and YAM primers/probe sets and the newly designed VER 2 VIC and YAM primers/probe sets was evaluated by testing each primers/probe set with influenza B viruse strains of the opposite lineage and from diverse geographic locations. Samples were extracted from high titer preparations of viruses ($\geq 10^6$ EID_{50}/mL) using the Roche MagNA Pure Compact RNA Isolation Kit. Cross-reactivity was evaluated with both enzyme systems FDA-cleared for use with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. No cross-reactivity was observed for any of the influenza B strains at the concentrations tested with any of the primers/probe sets (See Table 10 and Table 11 below).

Table 10: Analytical Exclusivity Testing #1 – VER 1.1 and VER 2 VIC Assays

Influenza Virus B/Yamagata Strain Designation	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™		Quanta qScript™	
		VIC (VER 1.1)	VIC (VER 2)	VIC (VER 1.1)	VIC (VER 2)
B/Brisbane/03/2007	$10^{8.2}$	-	-	-	-
B/Pennsylvania/07/2007	$10^{8.4}$	-	-	-	-
B/Hubei-Wujiagang/158/2009	$10^{6.2}$	-	-	-	-
B/Wisconsin/01/2010	$10^{8.5}$	-	-	-	-
B/Finland/39/2010	$10^{8.9}$	-	-	-	-
B/Estonia/55669/2011	$10^{8.4}$	-	-	-	-
B/Taiwan/1242/2011	$10^{9.1}$	-	-	-	-
B/Massachusetts/02/2012	$10^{6.3}$	-	-	-	-
B/Brisbane/03/2007	$10^{6.5}$	-	-	-	-
B/Pennsylvania/07/2007	$10^{4.2}$	-	-	-	-

Table 11: Analytical Exclusivity Testing #1 - VER 1.1 and VER 2 YAM Assays

Influenza Virus B/Victory Strain Designation	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™		Quanta qScript™		
		YAM (VER 1.1)	YAM (VER 2)	YAM (VER 1.1)	YAM (VER 2)	YAM (VER 1.1)
B/Hong Kong/259/2010	$10^{8.4}$	-	-	-		-
B/Bolivia/1526/2010	$10^{8.2}$	-	-	-		-
B/Laos/89/2011	$10^{8.2}$	-	-	-		-
B/Michigan/09/2011	$10^{8.9}$	-	-	-		-
B/New Jersey/01/2012	$10^{8.1}$	-	-	-		-
B/Montana/05/2012	$10^{7.8}$	-	-	-		-
B/Texas/02/2013	$10^{9.9}$	-	-	-		-
B/Florida/103/2016	$10^{9.2}$	-	-	-		-
B/Florida/76/2016	$10^{9.9}$	-	-	-		-
B/Hong Kong/269/2017	$10^{9.9}$	-	-	-		-

Potential cross-reactivity of the modified VER 1.1 VIC and YAM primers/probe sets and the newly designed VER 2 VIC and YAM primers/probe sets was also examined with influenza A viruses of various subtypes that circulate in humans and from animal origin that infect humans. Samples were prepared from characterized, high titer stocks ($\geq 10^6$ $TCID_{50}$/mL or EID_{50}/mL) by extracting RNA using the Roche MagNA Pure Compact RNA Isolation Kit. Testing was performed using both of the currently FDA-cleared enzyme systems. No cross-reactivity was observed for any of the influenza A strains at the concentrations tested with any of the primers/probe sets (See Table 12 below).

Table 12: Analytical Exclusivity Testing #2 – VER 1.1 and VER 2 VIC and YAM Assays

Influenza A Virus Strain Designation	Origin	Subtype	$TCID_{50}$/mL or EID_{50}/mL	Invitrogen SuperScript™				Quanta qScript™			
				VIC (VER 1.1)	VIC (VER 2)	YAM (VER 1.1)	YAM (VER 2)	VIC (VER 1.1)	VIC (VER 2)	YAM (VER 1.1)	YAM (VER 2)
A/Michigan/45/2015	Human	A(H1N1) pdm09	$10^{8.3}$	-	-	-	-	-	-	-	-
A/Hong Kong/4801/2014	Human	A(H3N2)	$10^{7.9}$	-	-	-	-	-	-	-	-
A/Ohio/35/2017	Swine	A(H1N2)v	$10^{6.9}$	-	-	-	-	-	-	-	-
A/Ohio/13/2017	Swine	A(H3N2)v	$10^{6.9}$	-	-	-	-	-	-	-	-

A/gyrfalcon/ Washington/ 41088-6/2014	Avian	A(H5N8)	$10^{9.75}$	-	-	-	-	-	-	-	-
A/Northern pintail/Washington/ 40964/2014	Avian	A(H5N2)	$10^{9.4}$	-	-	-	-	-	-	-	-
A/Bangladesh/ 0994/2011	Avian	A(H9N2)	$10^{10.5}$	-	-	-	-	-	-	-	-
A/Anhui/01/2013	Avian	A(H7N9)	$10^{10.9}$	-	-	-	-	-	-	-	-

Potential cross-reactivity of the newly designed VER 2 VIC and YAM primers/probe sets was also examined with non-influenza human respiratory viruses, bacteria, and yeast. Nucleic acids were extracted using the Roche MagNA Pure Compact RNA Isolation Kit from 36 organisms (16 viruses, 19 bacteria, and 1 yeast) representing common respiratory pathogens or flora commonly present in specimens collected from the nasopharynx region. All bacteria, yeast, and non-influenza viruses were from CDC repositories or acquired from American Type Culture Collection (ATCC, Manassas, VA) and tested at high titers, typically $\geq 10^6$ $TCID_{50}$/mL or EID_{50}/mL, $\geq 10^6$ CFU/mL, or as high as culture allowed. Testing was performed using both currently FDA-cleared enzyme systems. No cross-reactivity was observed for any of the organisms at the concentrations tested with any of the VER 2 primers/probe sets (See Table 13 below).

Table 13: Analytical Exclusivity Testing #2 – VER 2 VIC and YAM Assays

Organism Tested			Invitrogen SuperScript™		Quanta qScript™	
Bacteria and Yeast	Strain	CFU/mL	VIC (VER 2)	YAM (VER 2)	VIC (VER 2)	YAM (VER 2)
Bordetella pertussis	A639	$10^{8.3}$	-	-	-	-
Candida albicans	2001-21-196	$10^{8.8}$	-	-	-	-
Chlamydia pneumoniae[1]	TW183	40 IFU/mL	-	-	-	-
Corynebacterium diphtheriae	N/A	10^{10}	-	-	-	-
Escherichia coli	K12	$10^{9.6}$	-	-	-	-
Haemophilus influenzae	M15709	$10^{6.4}$	-	-	-	-
Lactobacillus plantarum	N/A	$10^{8.8}$	-	-	-	-
Legionella pneumophila	N/A	$10^{10.3}$	-	-	-	-
Moraxella catarrhalis	M15757	$10^{9.5}$	-	-	-	-
Mycobacterium tuberculosis[2]	H37Rv	95 ng/ µL	-	-	-	-
Mycoplasma pneumoniae	MI-29	$10^{7.7}$	-	-	-	-
Neisseria elongata	N/A	$10^{8.6}$	-	-	-	-
Neisseria meningitidis	M2578	$10^{7.9}$	-	-	-	-
Pseudomonas aeruginosa	N/A	$10^{10.5}$	-	-	-	-
Staphylococcus epidermidis	N/A	$10^{10.5}$	-	-	-	-
Staphylococcus aureus	N/A	$10^{10.7}$	-	-	-	-
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 or EID_{50}/mL	VIC (VER 2)	YAM (VER 2)	VIC (VER 2)	YAM (VER 2)

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]	N/A	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	$10^{6.9}$	-	-	-	-

N/A = Not Applicable

[1] Organism quantified by Infectious Forming Units (IFU)

[2] Organism quantified by spectrophotometry (ng/µL)

7. Clinical Performance

Retrospective Study - Influenza B (InfB) Assay

The clinical performance of the InfB assay oligonucleotide probe quenched with ZEN was evaluated using retrospective clinical samples collected during the 2011-2012 influenza season that were previously determined to be positive or negative for influenza B virus. A total of 30 positive and 50 negative upper respiratory tract samples were evaluated with the InfB assay using either the FDA-cleared oligonucleotide probe quenched with BHQ-1 or the oligonucleotide probe quenched with ZEN. Testing was performed with both enzymes FDA-cleared for use with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel and one of the FDA-cleared extraction methods. Result interpretation followed the instructions of the Package Insert for the FDA-cleared Influenza B Lineage Genotyping Kit. The InfB assay containing the oligonucleotide probe quenched with ZEN demonstrated 100% positive and negative agreement with the FDA-cleared oligonucleotide probe quenched with BHQ-1 (Table 14 and Table 15 below).

Table 14: InfB Assay (with the alternative ZEN quenched probe) Performance Testing Retrospective Positive Upper Respiratory Tract Clinical Specimens – Positive Percent Agreement vs. the Comparator (InfB assay with the BHQ-1 quenched probe)

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Positive Percent Agreement (95% CI)	# of Positives Correctly Identified vs. the Comparator	Positive Percent Agreement (95% CI)
NPS, NS	18/18	100% (82.4% - 100%)	18/18	100% (82.4% - 100%)
NPS/TS	11/11	100% (74.1% - 100%)	11/11	100% (74.1% - 100%)
NW	1/1	100% (20.7% - 100%)	1/1	100% (20.7% - 100%)

Total	30/30	100% (88.7% - 100%)	30/30	100% (88.7% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; NPS/TS = dual nasopharyngeal swab and throat swab; NW = nasal wash

Table 15: InfB Assay (with the alternative ZEN quenched probe) Performance Testing Retrospective Negative Upper Respiratory Tract Clinical Specimens – Negative Percent Agreement vs. the Comparator (InfB assay with the BHQ-1 quenched probe)

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type[1]	# of Negatives Correctly Identified vs. the Comparator	Negative Percent Agreement (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Negative Percent Agreement (95% CI)
NPS, NS	48/48	100% (92.6% - 100%)	48/48	100% (92.6% - 100%)
NPS/TS	2/2	100% (34.2% - 100%)	2/2	100% (34.2% - 100%)
Total	50/50	100% (92.9% - 100%)	50/50	100% (92.9% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; NPS/TS = dual nasopharyngeal swab and throat swab; NW = nasal wash

Prospective Study – VER 2 VIC and YAM Assays

To assess clinical performance of the newly designed VIC (VER 2) and YAM (VER 2) assays, a prospective clinical investigation was conducted at three U.S. public health laboratories using upper respiratory tract clinical specimens collected during the 2016-2017 influenza season. Samples were taken from specimens collected for routine influenza testing at each site from individuals symptomatic for influenza-like illness. The range of patient ages and specimens types for the total of 592 upper respiratory tract clinical specimens collected are represented in Table 16 below.

Table 16: Prospective Clinical Specimen Information

Patient Age	Totals
0-16	149
17-54	192
≥ 55	249
Not Reported	2
Total	592
Specimen Type[1]	Totals
NPS	374
NPS/TS	44
NA	12
NW	34
TS	13
NS	114
Not Reported	1
Total	592

[1] NPS = nasopharyngeal swab; NPS/TS = dual nasopharyngeal swab and throat swab; NA = nasal aspirate; NW = nasal wash; TS = throat swab; NS = nasal swab

Specimens were tested with assays from the FDA-cleared CDC Human Influenza Real-Time RT-PCR Diagnostic Panel (including the Influenza B Lineage Genotyping Kit) and the newly designed VIC (VER 2) and YAM (VER 2) assays. A total of 13 upper respiratory tract clinical

specimens were excluded from the performance analysis due to unspecified specimen type, inconclusive result of the comparator, or technician testing error. A total of 579 specimens were included in the performance analysis.

The performance of the newly designed VIC (VER 2) and YAM (VER 2) assays against the comparator (i.e., FDA-cleared Influenza B Lineage Genotyping Kit) is summarized in Table 17 and Table 18 below.

Table 17: VIC (VER 2) Assay Performance Testing Prospective Upper Respiratory Tract Clinical Specimens – Sensitivity and Specificity vs. the Comparator (FDA-cleared Influenza B Lineage Genotyping Kit)

Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Sensitivity (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Specificity (95% CI)
NPS, NS	5/5	100% (56.6% - 100%)	472/472	100% (99.2% - 100%)
NPS/TS	0/0	N/A	44/44	100% (92.0% - 100%)
TS	0/0	N/A	13/13	100% (77.2% - 100%)
NA, NW	0/0	N/A	45/45	100% (92.1% -100%)
Total	5/5	100% (56.6% - 100%)	574/574	100% (99.3% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; NPS/TS = dual nasopharyngeal swab and throat swab; NA = nasal aspirate; NW = nasal wash; TS = throat swab

Table 18: YAM (VER 2) Assay Performance Testing Prospective Upper Respiratory Tract Clinical Specimens – Sensitivity and Specificity vs. the Comparator (FDA-cleared Influenza B Lineage Genotyping Kit)

Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Sensitivity (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Specificity (95% CI)
NPS, NS	31/31	100% (89.0% - 100%)	446/446	100% (99.1% - 100%)
NPS/TS	2/2	100% (34.2% - 100%)	42/42	100% (91.6% - 100%)
TS	0/0	N/A	13/13	100% (77.2% - 100%)
NA, NW	5/5	100% (56.6% - 100%)	39/40	97.5% (87.1% -99.6%)
Total	38/38	100% (90.8% - 100%)	540/541	99.8% (99.0% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; NPS/TS = dual nasopharyngeal swab and throat swab; NA = nasal aspirate; NW = nasal wash; TS = throat swab

Retrospective Study – VER 2 VIC and YAM Assays

A retrospective study was performed using the VIC (VER 2) and the YAM (VER 2) assays to supplement the prospective performance assessment. Upper respiratory tract clinical samples were collected during the 2016-2017 and 2017- 2018 influenza seasons and determined to be positive or negative for influenza B/Victoria or B/Yamagata viruses using the FDA-cleared Influenza B Lineage Genotyping Kit. The specimens were tested using the FDA-cleared InfB assay and the newly designed VIC (VER 2) and YAM (VER 2) assays in this study. A total of 126 upper respiratory tract clinical specimens positive for either influenza B/Victoria or influenza B/Yamagataviruses and 61 specimens negative for influenza B viruses were tested. Result interpretation followed the instructions of the Package Insert for the FDA-cleared Influenza B Lineage Genotyping Kit.

The performance of the newly designed VIC (VER 2) and YAM (VER 2) assays against the comparator (i.e., FDA-cleared Influenza B Lineage Genotyping Kit) is summarized in Table 19 and Table 20 below.

Table 19: VIC (VER 2) Assay Performance Testing Retrospective Upper Respiratory Tract Clinical Specimens – Positive and Negative Agreement vs. the Comparator (FDA-cleared Influenza B Lineage Genotyping Kit)

Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Positive Percent Agreement (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Negative Percent Agreement (95% CI)
NPS, NS	29/29	100% (88.3% - 100%)	156/156	100% (97.6% - 100%)
TS	1/1	100% (20.7% - 100%)	1/1	100% (20.7% - 100%)
Total	30/30	100% (88.7% - 100%)	157/157	100% (97.6% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; TS = throat swab

Table 20: YAM (VER 2) Assay Performance Testing Retrospective Upper Respiratory Tract Clinical Specimens – Positive and Negative Agreement vs. the Comparator (FDA-cleared Influenza B Lineage Genotyping Kit)

Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Positive Percent Agreement (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Negative Percent Agreement (95% CI)
NPS, NS	95/95	100% (96.1% - 100%)	90/90	100% (95.9% - 100%)
TS	1/1	100% (20.7% - 100%)	1/1	100% (20.7% - 100%)
Total	96/96	100% (96.2% - 100%)	91/91	100% (96.0% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; TS = throat swab

Retrospective Study – VER 1.1 VIC and YAM Assays

A retrospective study was performed using the VIC (VER 1.1) and the YAM (VER 1.1) assays to assess performance of the modified VIC and YAM assays. Upper respiratory tract clinical samples were collected during the 2016-2017 and 2017- 2018 influenza seasons and determined to be positive or negative for influenza B/Victoria or B/Yamagata viruses using the FDA-cleared Influenza B Lineage Genotyping Kit. The specimens were tested using the FDA-cleared InfB assay and the modifed VIC (VER 1.1) and YAM (VER 1.1) assays in this study. A total of 126 upper respiratory tract clinical specimens positive for either influenza B/Victoria or influenza B/Yamagata viruses and 61 specimens negative for influenza B viruses were tested. Result interpretation followed the instructions of the Package Insert for the FDA-cleared Influenza B Lineage Genotyping Kit.

The performance of the modified VIC (VER 1.1) and YAM (VER 1.1) assays against the comparator (i.e., FDA-cleared Influenza B Lineage Genotyping Kit) is summarized in Table 21 and Table 22 below.

Table 21: VIC (VER 1.1) Assay Performance Testing Retrospective Upper Respiratory Tract Clinical Specimens – Positive and Negative Agreement vs. the Comparator (FDA-cleared Influenza B Lineage Genotyping Kit)

Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Positive Percent Agreement (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Negative Percent Agreement (95% CI)
NPS, NS	29/29	100% (88.3% - 100%)	156/156	100% (97.6% - 100%)
TS	1/1	100% (20.7% - 100%)	1/1	100% (20.7% - 100%)
Total	30/30	100% (88.7% - 100%)	157/157	100% (97.6% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; TS = throat swab

Table 22: YAM (VER 1.1) Assay Performance Testing Retrospective Upper Respiratory Tract Clinical Specimens – Positive and Negative Agreement vs. the Comparator (FDA-cleared Influenza B Lineage Genotyping Kit)

Specimen Type[1]	# of Positives Correctly Identified vs. the Comparator	Positive Percent Agreement (95% CI)	# of Negatives Correctly Identified vs. the Comparator	Negative Percent Agreement (95% CI)
NPS, NS	95/95	100% (96.1% - 100%)	90/90	100% (95.9% - 100%)
TS	1/1	100% (20.7% - 100%)	1/1	100% (20.7% - 100%)
Total	96/96	100% (96.2% - 100%)	91/91	100% (96.0% - 100%)

[1] NPS = nasopharyngeal swab; NS = nasal swab; TS = throat swab

8. Conclusion

The labeling for this modified subject device has been reviewed to verify that the indication/intended use for the device is unaffected by the modifications. In addition, the applicant's description of the particular modification(s) and the comparative information between the modified and unmodified devices demonstrate that the fundamental scientific technology has not changed. The applicant has provided the design control information as specified in The New 510(k) Paradigm and on this basis, I recommend the device be determined substantially equivalent to the predicate device.