



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY**

**I Background Information:**

**A 510(k) Number**

K243931

**B Applicant**

Centers for Disease Control and Prevention

**C Proprietary and Established Names**

CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit (VER 2); Influenza A Subtyping Kit (VER 4); Influenza B Lineage Genotyping Kit (VER 1.1 and 2); and Influenza A/H5 Subtyping Kit (VER 4)

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
OZE	Class II	21 CFR 866.3980 - Respiratory Viral Panel Multiplex Nucleic Acid Assay	MI - Microbiology

**II Submission/Device Overview:**

**A Purpose for Submission:**

The purpose of this submission is to obtain FDA-clearance for the modification of the Influenza A/H5 Subtyping Kit, one of the four component assay kits of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel, to add conjunctival swabs as an acceptable specimen type.

**B Measurand:**

Influenza A and B nucleic acids

**C Type of Test:**

Molecular Real-Time Reverse Transcription - Polymerase Chain Reaction (RT-PCR) tests

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

See Indications for Use below.

#### **B Indication(s) for Use:**

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit (VER 2)

The Influenza A/B Typing Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:

- For qualitative detection of influenza virus type A or B viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture.
- To provide epidemiological information for surveillance of circulating influenza viruses.

Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A Subtyping Kit (VER 4)

The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:

- For determination of the subtype of seasonal human influenza A viruses as seasonal A(H3) and/or A(H1)pdm09 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate

[TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;

- To provide epidemiological information for surveillance of circulating influenza viruses.

Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

#### CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2)

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 in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit 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 in circulation.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

#### CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit (VER 4)

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 in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit 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, conjunctival swabs, and viral culture in conjunction with clinical and epidemiological risk factors;
- To provide epidemiologic information for surveillance of circulating influenza viruses.

Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.

Testing with the influenza H5a and H5b primer and probe sets should not be performed unless the patient meets the most current U.S. Department of Health and Human Services (DHHS) clinical and epidemiologic criteria for testing suspect A(H5) specimens. The definitive identification of influenza A(H5) (Asian lineage) either directly from patient specimens or from virus cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not

rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

#### **C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only  
For *In Vitro* Diagnostic Use

#### **D Special Instrument Requirements:**

*In vitro* diagnostic real-time PCR instrument that has been FDA-cleared for use with this panel

### **IV Device/System Characteristics:**

#### **A Device Description:**

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is used in real-time RT-PCR (rRT-PCR) assays on an *in vitro* diagnostic real-time PCR system that has been FDA-cleared for use with this panel. The panel is configured in four separate assay kits: Influenza A/B Typing Kit, Influenza A Subtyping Kit, Influenza B Lineage Genotyping Kit, and Influenza A/H5 Subtyping Kit.

Each assay 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 mainly respiratory specimens from patients presenting with influenza-like illness (ILI). Oligonucleotide primers and probes for detection of influenza A, influenza B, and 2009 influenza A (swine origin) were selected from highly conserved regions of the matrix (M), non-structural (NS), and nucleoprotein (NP) genes, respectively. Oligonucleotide primers and probes for characterization and differentiation of influenza A(H3) and A(H1)pdm09 viruses and genetic lineages of influenza B were selected from highly conserved regions of their HA genes. Oligonucleotide primers and probes for characterization and differentiation of avian influenza A(H5) (Asian lineage) viruses were also selected from highly conserved regions of the HA genes. Oligonucleotide primers and probes to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

## B Principle of Operation:

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is a nucleic acid amplification test that employs real-time RT-PCR (rRT-PCR) assays that detect influenza A and B viruses, and further characterize influenza A subtypes A(H1)pdm09, A(H3), and A(H5) (Asian lineage) and influenza B lineages B/Victoria and B/Yamagata.

## V Substantial Equivalence Information:

### A Predicate Device Name(s):

CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit, Influenza A Subtyping Kit (VER 2), Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2), and Influenza A/H5 Subtyping Kit (VER 3)

### B Predicate 510(k) Number(s):

K190302

### C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K243931</u>	<u>K190302</u>
Device Trade Name	CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit (VER 2), Influenza A Subtyping Kit (VER 4), Influenza B Lineage Genotyping Kit (VER 1.1 and 2), and Influenza A/H5 Subtyping Kit (VER 4)	CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit, Influenza A Subtyping Kit (VER 2), Influenza B Lineage Genotyping Kit (VER 1.1 and 2), and Influenza A/H5 Subtyping Kit (VER 3)
General Device Characteristic Similarities		
Intended Use/Indications For Use	Same, except for the addition of a conjunctival swab specimen type to the Intended Use/Indications For Use of the Influenza A/H5 Subtyping Kit only.	CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit  The Influenza A/B Typing Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:

		<ul style="list-style-type: none"> <li>• For qualitative detection of influenza virus type A or B viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture.</li> <li>• To provide epidemiological information for surveillance of circulating influenza viruses.</li> </ul> <p>Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.</p> <p>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.</p> <p>If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E</p>
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		<p>facility is available to receive and culture specimens.</p> <p>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.</p> <p>CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A Subtyping Kit (VER 2)</p> <p>The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:</p> <ul style="list-style-type: none"> <li>• For determination of the subtype of seasonal human influenza A viruses as seasonal A(H3) and/or A(H1)pdm09 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;</li> <li>• To provide epidemiological information for surveillance of circulating influenza viruses.</li> </ul>
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		<p>Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.</p> <p>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.</p> <p>If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.</p> <p>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.</p> <p>CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza B Lineage Genotyping Kit (VER 1.1 and VER 2)</p>
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		<p>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 in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:</p> <ul style="list-style-type: none"> <li>• 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;</li> <li>• To provide epidemiologic information for surveillance of circulating influenza viruses.</li> </ul> <p>Performance characteristics for influenza B lineage genotyping were established during a season when influenza B/Victoria and B/Yamagata lineages were in circulation.</p> <p>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.</p> <p>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</p>
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		<p>training course provided by CDC instructors or designees.</p> <p>CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit (VER 3)</p> <p>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 in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:</p> <ul style="list-style-type: none"> <li>• 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, conjunctival swabs, and viral culture in conjunction with clinical and epidemiological risk factors;</li> <li>• To provide epidemiologic information for surveillance of circulating influenza viruses.</li> </ul> <p>Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.</p> <p>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</p>
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		<p>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.</p> <p>Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.</p> <p>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.</p> <p>If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.</p> <p>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.</p>
Analyte	Same	Influenza RNA
Technological Principles	Same	Real-time RT-PCR
<b>General Device Characteristic Differences</b>		
Specimen Type	For the Influenza A/H5 Subtyping Kit only: human respiratory specimens,	Human respiratory specimens and viral culture

	conjunctival swabs, and viral culture	
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## VI Standards/Guidance Documents Referenced:

None

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

#### 1. Precision/Reproducibility:

No new precision/reproducibility data were reviewed in this 510(k). The only modification to the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel in this 510(k) submission is the addition of a conjunctival swab specimen type for use with the Influenza A/H5 Subtyping Kit, which is one of the four component assay kits of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Refer to FDA Decision Summaries (K200370, K190302, K172091, K153148, K141859, K130551, K111507, and K080570) for precision/reproducibility data FDA reviewed and accepted previously.

#### 2. Linearity:

Not Applicable. This is a qualitative test.

#### 3. Analytical Specificity/Interference:

No new analytical specificity data were reviewed in this 510(k). The only modification to the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel in this 510(k) submission is the addition a conjunctival swab specimen type for use with the Influenza A/H5 Subtyping Kit, which is one of the four component assay kits of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Refer to FDA Decision Summaries (K200370, K190302, K172091, K153148, K141859, K130551, K111507, and K080570) for analytical specificity data FDA reviewed and accepted previously.

Two new studies were performed to evaluate the potential impact of exogenous interfering substances in conjunctival swab specimen matrix. Studies were conducted testing two common over the counter eye drops containing olopatadine hydrochloride or naphazoline hydrochloride.

For the first study, to evaluate the effect of the eyedrops on the assay, a set of contrived specimens (N=5) was constructed with a measured volume of each eyedrop plus influenza A/American Wigeon/South Carolina/22-000345-001/2021 H5N1 at 2x LoD in conjunctival swab matrix. An additional set of contrived specimens (N=5) was constructed with a measured volume of each eyedrop plus influenza A/American Wigeon/South Carolina/22-000345-001/2021 H5N1 at 2x LoD in VTM and A549 cells (a simulated respiratory matrix in

VTM). Each contrived specimen contains 2.31% Clear Eyes eyedrop (naphazoline hydrochloride as the main ingredient) and 2.08% Pataday eyedrop (olopatadine hydrochloride as the main ingredient), and was extracted on the Qiagen EZ1 Advanced XL with the EZ1 DSP Virus kit, amplified by the Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR System (without Rox) and run on the Applied Biosystems 7500 Fast Dx PCR platform. Due to a lack of sufficient volume of natural conjunctival swabs, the study was conducted by mixing both the olopatadine hydrochloride and naphazoline hydrochloride into a single reaction in this study. Cycle threshold (Ct) results were compared to contrived specimens tested at 2x LoD without the addition of eyedrops as the control condition. No interference was observed with the addition of olopatadine hydrochloride and naphazoline hydrochloride at the concentration tested in either the contrived specimens in natural conjunctival matrix or the contrived specimens in the simulated respiratory matrix. The results of this study are presented below.

**Table 1. Interfering Substance Study Results - Evaluation of Eyedrops (Olopatadine Hydrochloride and Naphazoline Hydrochloride) in Conjunctival Swab Specimen Matrix and Simulated Respiratory Matrix**

Matrix	Interfering Substance Testing Condition	Replicates Tested	A/H5 Subtyping Kit Assay Target							
			InfA		H5a		H5b		RP	
			Avg Ct	St Dev	Avg Ct	St Dev	Avg Ct	St Dev	Avg Ct	St Dev
Conjunctival Swab Matrix	Without Eyedrops	5	29.49	0.40	33.81	0.31	33.22	0.63	26.72	0.23
	With Eyedrops	5	29.35	0.37	33.50	0.61	33.12	0.31	26.36	0.21
Simulated Respiratory Matrix	Without Eyedrops	5	29.35	0.79	33.47	0.89	33.38	0.40	27.72	1.17
	With Eyedrops	5	28.26	0.33	32.24	0.31	32.73	0.60	27.16	0.17

The second study was performed by adding 5 µl of Pataday (olopatadine hydrochloride as the main ingredient) or Clear Eyes (naphazoline hydrochloride as the main ingredient) eyedrop dilutions in molecular-grade-water (2.22% Pataday and 2.46% Clear Eyes, respectively) directly into the RT-PCR enzyme master mix and tested in 20 replicates to evaluate the effect of the eyedrop chemistry on the A/H5 assay and determine if autofluorescence is produced due to the eyedrop additions that could generate a false positive A/H5 assay result. No false positive A/H5 assay result was observed when the eyedrops were added directly to the RT-PCR reactions in this study. The results of this study are presented below.

**Table 2. Interfering Substance Study Results - Evaluation of Eyedrops (Olopatadine Hydrochloride or Naphazoline Hydrochloride) Added Directly to the RT-PCR Reaction**

Replicate	Olopatadine Hydrochloride Added Directly to the RT-PCR Reactions				Naphazoline Hydrochloride Added Directly to the RT-PCR Reactions			
	Ct Values				Ct Values			
	InfA	H5a	H5b	RP	InfA	H5a	H5b	RP
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

4. Assay Reportable Range:

Not Applicable. This is a qualitative test.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

No new data were reviewed in this 510(k). The only modification to the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel in this 510(k) submission is the addition a conjunctival swab specimen type for use with the Influenza A/H5 Subtyping Kit, which is one of the four component assay kits of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Refer to FDA Decision Summaries (K200370, K190302, K172091, K153148, K141859, K130551, K111507, and K080570) for more details.

6. Detection Limit:

No new limit of detection data from testing respiratory specimens were reviewed in this 510(k). The only modification to the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel in this 510(k) submission is the addition a conjunctival swab specimen type for use with the Influenza A/H5 Subtyping Kit, which is one of the four component assay kits of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Refer to FDA Decision Summaries (K200370, K190302, K172091, K153148, K141859, K130551, K111507, and K080570) for limit of detection data for respiratory tract specimens FDA reviewed and accepted previously.

To assess the Limit of Detection (LoD) of the Influenza A/H5 Subtyping Kit testing conjunctival swab collected in VTM/UTM specimens, an initial LoD range-finding experiment using  $\beta$ -Propiolactone (BPL) inactivated influenza A/American

Wigeon/South Carolina/22-000345- 001/2021 H5N1 was performed in a background of VTM and A549 human lung epithelial cells (a simulated respiratory matrix in VTM). A 5-fold dilution series of the virus was prepared using this simulated matrix as the diluent. Each dilution step was tested in triplicate using the CDC Flu rRT-PCR Dx Panel Influenza A/H5 Subtyping kit. The range finding limit of detection was defined as the viral titer of the last dilution step demonstrating 100% positive results (3/3) in this experiment. Results demonstrated a preliminary LoD with an approximate titer of  $10^{5.5}$  EID<sub>50</sub>/ml.

Due to very limited availability of negative conjunctival swab matrix material, the estimated preliminary LoD was confirmed by testing replicates at concentrations of 2x and 5x of preliminary LoD using contrived specimens in either influenza A/H5 negative conjunctival swab matrix or the simulated respiratory matrix in VTM. Contrived specimens comprised of either negative conjunctival swab matrix and BPL inactivated influenza A/American Wigeon/South Carolina/22-000345-001/2021 H5N1 virus or negative simulated respiratory matrix in VTM and BPL inactivated influenza A/American Wigeon/South Carolina/22-000345-001/2021 H5N1 virus were constructed at 2x and 5x of the estimated preliminary LoD. Five replicates of each contrived specimen type at either 2x LoD or 5x LoD were tested along with 10 negative specimens for each matrix. Each sample was extracted on the Qiagen EZ1 Advanced XL with the EZ1 DSP Virus kit, amplified by the Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR System (without Rox) and run on the Applied Biosystems 7500 Fast Dx PCR platform. Detection levels and Ct values of the conjunctival swab matrix were similar to that observed in the simulated respiratory matrix in VTM at both the 2x and 5x LoD concentrations. Based on this data, the LoD for the conjunctival swab collected in VTM/UTM specimens was determined to be  $10^{5.5}$  EID<sub>50</sub>/ml.

**Table 3. Limit of Detection (LoD) - Conjunctival Swab in VTM/UTM**

Concentration	Matrix Sample	Replicates Tested	A/H5 Subtyping Kit Assay Target							
			InfA		H5a		H5b		RP	
			Avg Ct	St Dev	Avg Ct	St Dev	Avg Ct	St Dev	Avg Ct	St Dev
2x $10^{5.5}$ EID <sub>50</sub> /ml	Conjunctival Swab Matrix	5	29.49	0.40	33.81	0.31	33.22	0.63	26.72	0.23
	Simulated Respiratory Matrix in VTM	5	29.35	0.79	33.47	0.89	33.38	0.40	27.72	1.17
5x $10^{5.5}$ EID <sub>50</sub> /ml	Conjunctival Swab Matrix	5	26.77	0.19	31.06	0.25	30.91	0.37	26.54	0.28
	Simulated Respiratory Matrix in VTM	5	26.26	0.26	30.37	0.20	30.76	0.20	26.77	0.23
Negative	Conjunctival Swab Matrix	10	0.00	0.00	0.00	0.00	0.00	0.00	25.76	0.24
	Simulated Respiratory Matrix in VTM	10	0.00	0.00	0.00	0.00	0.00	0.00	27.86	0.43

EID<sub>50</sub> - Egg Embryo Infectious Dose

## 7. Assay Cut-Off:



Refer to FDA Decision Summaries (K200370, K190302, K172091, K153148, K141859, K130551, K111507, and K080570) for assay cut-offs FDA reviewed and accepted previously.

## **B Comparison Studies:**

### 1. Method Comparison with Predicate Device:

Not applicable. See section C “Clinical Studies” for clinical validation data supporting the addition of a conjunctival swab specimen type for use with the Influenza A/H5 Subtyping Kit, which is one of the four component assay kits of the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel.

### 2. Matrix Comparison:

Not Applicable.

## **C Clinical Studies:**

### 1. Clinical Sensitivity:

Clinical sensitivity has not been assessed due to the lack of an FDA-cleared comparator for A/H5 specific detection and subtyping from conjunctival swabs collected in VTM/UTM specimens.

### 2. Clinical specificity:

Clinical Specificity has not been assessed due to the lack of an FDA-cleared comparator for A/H5 specific detection and subtyping from conjunctival swabs collected in VTM/UTM specimens.

### 3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Human upper respiratory tract (URT) swab specimens, including nasopharyngeal swab (NPS) specimens, combined nasal swab (NS) and oropharyngeal swab (OS) specimens, NS and/or OS specimens, as well as human conjunctival swab (CS) specimens were collected by health care professionals (HCPs) as part of the public health investigations into the outbreaks of influenza A/H5 in dairy cows and poultry in the US from March to November 2024. All available URT swab specimens and conjunctival swab (CS) specimens from individuals with either a presumptive influenza A/H5 positive laboratory result (in any specimen) at the state public health laboratories, or a strong epidemiological link and clinical suspicion that indicate suspected influenza A/H5 infection, were sent to the CDC laboratory in Atlanta for confirmatory testing and evaluation.

URT swab specimens and CS specimens collected from a total of 44 confirmed and two probable human cases of A/H5N1 from March to November of 2024 were received and tested using the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Influenza A/H5 Subtyping Kit (VER 4) (i.e., the CDC A/H5 assay) at the CDC laboratory in Atlanta. The influenza A/H5N1 confirmed or probable case status for each case was

adjudicated based on the criteria described in Table VII.A. Classification Table: Criteria for defining a case of novel influenza A infection included in the Council of State and Territory Epidemiologists (CSTE) document 24-ID-09, which is titled “Update to Public Health Reporting and National Notification for Novel Influenza A Virus Infection”

[http://www.cste.org/resource/resmgr/position\\_statements\\_files\\_2023/24-ID-09\\_Novel\\_Influenza\\_A.pdf](http://www.cste.org/resource/resmgr/position_statements_files_2023/24-ID-09_Novel_Influenza_A.pdf)

Of the 44 confirmed human cases and two probable cases of A/H5N1, 42 (95.5%) and two (100%), respectively, reported conjunctivitis symptoms. While 28 confirmed cases and one probable case reported both respiratory and/or systemic symptoms and conjunctivitis symptoms, 16 confirmed cases and one probable case reported conjunctivitis symptoms only.

The mean age of the 46 confirmed and probable cases was 36.5 years (range: 18-57 years).

Due to a field correction of a manufacturing error of the H5b component assay reagents required for testing and result interpretation using the CDC A/H5 assay, clinical specimens from nine of the 44 confirmed cases were tested using the CDC A/H5 assay without the H5b component assay reagents in June and July of 2024. As a result, clinical specimens from these nine confirmed cases were excluded from performance assessment for the CDC A/H5 assay (i.e., the remaining 35 confirmed cases and two probable cases were included in the performance assessment).

Of the 35 confirmed cases assessed, 33/35 (94.3%) reported conjunctivitis symptoms. Of those 33 confirmed cases with conjunctivitis symptoms reported, 26/33 (78.8%) reported a positive result for A/H5 and 3/33 (9.1%) reported an inconclusive result for A/H5 on CS specimens using the CDC A/H5 assay. Four of the 33 confirmed cases with conjunctivitis symptoms (15.2%) reported a positive result for A/H5 on an NPS specimen only using the CDC A/H5 assay.

Of the two confirmed cases without conjunctivitis symptoms reported, 1/2 (50%) tested positive for A/H5 on a CS specimen, and 1/2 (50%) tested positive for A/H5 on an NS specimen only using the CDC A/H5 assay.

Of the two probable cases with conjunctivitis symptoms reported, 2/2 (100%) tested negative on CS specimens using the CDC A/H5 assay.

Subject matched paired URT swab specimens (i.e., NPS, combined NS and OS, NS, and/or OS) and CS specimens were available for testing in 32 out of 35 confirmed cases and one of the two probable cases of A/H5N1. For three of the 35 confirmed cases and one of the two probable cases, only CS specimens were available for testing.

Of the subject matched paired URT swab and CS specimens from 32 confirmed human cases of A/H5N1, the CDC A/H5 assay was positive on paired URT swab (at least one URT swab specimen is positive) and CS specimens in ten confirmed human cases (five paired NPS, four paired combined NS and OS, and one paired NPS and combined NS and OS specimens were positive in addition to the paired CS specimens being positive); the CDC A/H5 assay was positive in CS specimens only in 15 confirmed cases (two paired combined NS and OS and one paired NS specimens in three confirmed cases generated an A/H5 inconclusive result and all other paired URT swab specimens generated a negative result); and the CDC A/H5 assay

was positive in at least one URT swab specimen only in four confirmed cases (three paired NPS and one paired NS specimens were positive while all paired CS specimens were negative). For three of the 32 confirmed human cases of A/H5N1 where subject matched paired URT swab and CS specimens were available for testing, the CDC A/H5 assay generated an A/H5 inconclusive result on paired CS specimens only while all paired URT swab specimens were negative.

Of the subject matched paired URT swab and CS specimens from one probable human case of A/H5N1, the CDC A/H5 assay was negative on all paired URT swab (one NPS and one combined NS and OS specimens) and the CS specimen in this probable case.

For three of the 35 confirmed cases where only CS specimens were available for testing, the CDC A/H5 assay was positive on the CS specimens in two confirmed cases, and inconclusive in 1 confirmed case.

For one of the two probable cases where only a CS specimen was available for testing, the CDC A/H5 assay generated an A/H5 negative result.

Comparisons of subject-matched paired conjunctival swab (CS) and upper respiratory tract (URT) swab specimens (i.e., NPS, combined NS and OS, NS, and/or OS) testing results using the CDC A/H5 assay in confirmed and probable A/H5N1 cases (n=33) across all symptoms are presented in the two tables below.

**Table 4. Comparison of Subject Matched Paired URT Swab Specimens**

	Patient Symptoms			
	Conjunctivitis Only	Conjunctivitis + Flu-like Symptoms	Flu-like Symptoms Only	All Symptoms
A/H5 Positive CS Only	8	6	1	15
A/H5 Positive URT Swab Only	1	2	1	4
Positive in Both CS and URT Swab	2	8	0	10
Positive in Neither CS or URT Swab	1	3	0	4
Total Patients	12	19	2	33

CS - Conjunctival Swab

URT - Upper Respiratory Tract Swab

**Table 5. Comparison of Subject Matched Paired URT Swab Specimens**

	Patient Symptoms			
	Conjunctivitis Only	Conjunctivitis + Flu-like Symptoms	Flu-like Symptoms Only	All Symptoms

A/H5 Positive CS	10/12	14/19	1/2	25/33
A/H5 Positive URT Swab	3/12	10/19	1/2	14/33

CS - Conjunctival Swab

UTR - Upper Respiratory Tract Swab

**D Clinical Cut-Off:**

Not Applicable.

**E Expected Values/Reference Range:**

Refer to FDA Decision Summaries (K200370, K190302, K172091, K153148, K141859, K130551, K111507, and K080570) for Expected Values FDA reviewed and accepted previously.

**VIII Proposed Labeling:**

The labeling supports the finding of substantial equivalence for this device.

**IX Conclusion:**

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