

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

**A. 510(k) Number:**

K142365

**B. Purpose for Submission:**

The Simplexa™ Flu A/B & RSV Direct, originally cleared under k120413, was modified to improve detection of newer circulating influenza strains while maintaining sensitivity for detection of previous influenza strains. The labeling was updated to include analytical reactivity data for the following additional six (6) strains of influenza A and two (2) strains of influenza B: influenza A/Anhui/1/2013 (H7N7), influenza A/California/12/2012 (H1N1) pdm09, influenza A/Indiana/08/2011 (H3N2)v, influenza A/Minnesota/11/2010 (H3N2)v, influenza A/Ohio/02/2012 (H3N2), influenza A/Texas/50/2012(H3N2), influenza B/Brisbane/60/2008, and influenza B/Wisconsin/01/2010.

**C. Measurand:**

The Simplexa™ Flu A/B & RSV Direct detects target RNA sequences for the highly conserved regions of the matrix protein genes of influenza A and influenza B viruses, and the M gene of RSV.

**D. Type of Test:**

This is a qualitative Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay used with the 3M Integrated Cyclor instrument for the *in vitro* qualitative detection and differentiation of influenza A virus, influenza B virus and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from symptomatic patients. The assay measures viral RNA directly from un-extracted nasopharyngeal swab specimens. An on-board pre-heating step opens the viral coat releasing the viral RNA. A bi-functional fluorescent probe-primer is used together with a reverse primer to amplify a specific target for each analyte and the RNA internal control. Amplification and detection is performed on the 3M Integrated Cyclor with Integrated Cyclor Studio Software version 4.2 or higher.

**E. Applicant:**

Focus Diagnostics, Inc.

**F. Proprietary and Established Names:**

Simplexa™ Flu A/B & RSV Direct

Simplexa™ Flu A/B & RSV Positive Control Pack

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.3980 Respiratory viral panel multiplex nucleic acid assay

2. Classification:

Class II

3. Product code:

OCC, OOI

4. Panel:

Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

Simplexa™ Flu A/B & RSV Direct (REF MOL2650)

The Focus Diagnostics Simplexa™ Flu A/B & RSV Direct assay is intended for use on the 3M Integrated Cycler instrument for the in vitro qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors. This test is intended for use as an aid in the differential diagnosis of influenza A, influenza B, and RSV viral infections in humans and is not intended to detect influenza C.

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

Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009 H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

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 the state or local health department for testing. Viral culture

should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Simplexa™ Flu A/B & RSV Positive Control Pack (REF MOL2660)

Focus Diagnostics' Simplexa™ Flu A/B & RSV Positive Control Pack is intended to be used as a control with the Simplexa™ Flu A/B & RSV Direct kit. This control is not intended for use with other assays or systems.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For Prescription Use Only

4. Special instrument requirements:

3M Integrated Cycler with Integrated Cycler Studio Software version 4.2 or higher

**I. Device Description:**

See k120413 for full description of the device.

The Simplexa™ Flu A/B & RSV Direct (K120413) was modified to improve the detection of newer, evolving strains of influenza viruses. The changes were made in two phases. The first phase of the modifications (Gen 2.0) included changes to the reaction mix formulation and cycling conditions: (a) change in the annealing temperature (decreased), (b) change in the run time (increased), (c) change in the enzyme used, and (d) change to the RSV cut-off Ct (decreased). Limited changes (Gen. 2.1) were also made to the manufacturing process and materials to increase the stability of the reaction mix and to minimize non-specific products.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Simplexa™ Flu A/B & RSV Direct

2. Predicate 510(k) number(s):

K120413

3. Comparison with predicate:

The following shows the comparison (similarities and differences) of the modified device to applicant's legally marketed predicate device:

<b>Feature</b>	<b>Predicate K120413</b>	<b>Modified Device K142365</b>
Intended Use	<p>The Focus Diagnostics Simplexa™ Flu A/B &amp; RSV Direct assay is intended for use on the 3M Integrated Cycler instrument for the in vitro qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors. This test is intended for use as an aid in the differential diagnosis of influenza A, influenza B, and RSV viral infections in humans and is not intended to detect influenza C.</p> <p>Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.</p> <p>Focus Diagnostics' Simplexa™ Flu A/B &amp; RSV Positive Control Pack is intended to be used as a control with the Simplexa™ Flu A/B &amp; RSV Direct kit. This control is not intended for use with other assays or systems.</p>	SAME
Technology	<p>The Simplexa™ Flu A/B &amp; RSV Direct assay system is a real-time RT-PCR system that enables the direct amplification, detection and differentiation of human influenza A (Flu A) virus RNA, human influenza B (Flu B) virus RNA, and RSV RNA from unprocessed nasopharyngeal swabs that have not undergone nucleic acid extraction. The system consists of the Simplexa™ Flu A/B &amp; RSV Direct assay, the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct</p>	SAME

	Amplification Disc, and associated accessories. In the Simplexa™ Flu A/B & RSV Direct assay, bi-functional fluorescent probe-primers are used together with corresponding reverse primers to amplify Flu A, Flu B, RSV, and internal control RNA. An RNA internal control is used to detect RT-PCR failure and/or inhibition.	
Instrument	3M™ Integrated Cycler	SAME
Analyte	Human influenza A (Flu A) virus RNA, Human influenza B (Flu B) virus RNA RSV RNA	SAME
Assay Type	Qualitative	SAME
Analytical Reactivity	<ul style="list-style-type: none"> <li>• Influenza A Brisbane/10/07 H3,</li> <li>• Influenza A Brisbane/59/07 H1,</li> <li>• Influenza A New Caledonia/20/99 H1N1,</li> <li>• Influenza A Port Chalmers/1/73 H3N2,</li> <li>• Influenza A Solomon Island/03/06 H1,</li> <li>• Influenza A Taiwan/42/06 H1N1,</li> <li>• Influenza A Wisconsin/67/05 H3,</li> <li>• Influenza A WS/33 H1N1,</li> <li>• Influenza A/California/7/2009 NYMC x-179-A ,</li> <li>• Tissue Culture Adapted Influenza A/Swine H1N1/Iowa/15/1930,</li> <li>• Tissue Culture Adapted Influenza A Swine H1N1/USA/1976/1931,</li> <li>• Influenza A PR8 Vietnam/1203/2004 (H5N1 - inactivated virus),</li> <li>• Influenza B Allen/45,</li> <li>• Influenza B Florida/02/2006,</li> <li>• Influenza B Florida/04/2006,</li> <li>• Influenza B Florida/07/04,</li> <li>• Influenza B Hong Kong/5/72,</li> <li>• Influenza B Lee/40,</li> <li>• Influenza B Maryland/1/59,</li> <li>• Influenza B Panama/45/90,</li> <li>• Influenza B Taiwan/2/62,</li> </ul>	<p>SAME</p> <p>Plus additional 8 influenza strains:</p> <ul style="list-style-type: none"> <li>• Influenza A/California/12/2012 (H1N1) pdm09,</li> <li>• Influenza A/Ohio/02/2012 (H3N2),</li> <li>• Influenza A/Texas/50/2012 (H3N2),</li> <li>• Influenza A/Anhui/1/2013 (H7N9),</li> <li>• Influenza A/Minnesota/11/2010 (H3N2)v,</li> <li>• Influenza A/Indiana/08/2011 (H3N2)v,</li> <li>• Influenza B/Brisbane/60/2008,</li> <li>• Influenza B/Wisconsin/01/2010.</li> </ul>
Influenza A Viral Target	Well conserved region of the matrix gene	SAME

Influenza B Viral Target	Well conserved region of the matrix gene	SAME
RSV Target	M gene	SAME

**K. Standard/Guidance Document Referenced (if applicable):**

N/A

**L. Test Principle:**

The Simplexa™ Flu A/B & RSV is a real-time RT-PCR amplification and detection system that utilizes a bi-functional fluorescent probe-primer for the detection and differentiation of human influenza A virus RNA, human influenza B virus RNA and respiratory syncytial virus (RSV) RNA directly from unextracted nasopharyngeal swab (NPS) specimens. An on-board pre-heating step opens the viral coat releasing the viral RNA. A bi-functional fluorescent probe-primer is used together with a reverse primer to amplify a specific target (for each analyte and the RNA internal control). A fluorescent signal is generated as a result of the binding of a probe element to the extended DNA fragment synthesized during the amplification. The 3M Integrated Cycler is a thermocycler capable of heating, cooling, mixing of sample and reagents, amplification, and real-time detection of fluorescence. There are four optical channels allowing for detection of up to four targets simultaneously. The table below shows the excitation and emission wavelengths for each of the standard channels provided as part of the Integrated Cycler instrument.

Target	Probe Fluorophore (Dye)	Excitation $\lambda$	Emission $\lambda$
Flu A – matrix gene	FAM	495	520
Flu B – matrix gene	JOE	520	548
RSV – M gene	CFR610	590	610
Internal Control	Q670	644	670

**M. Performance Characteristics:**

1. Analytical performance:

See k120413

The Simplexa Flu A/B and RSV Direct assay was modified in two phases (Gen. 2.0 and Gen 2.1). The performance of the device was evaluated in analytical studies after each modification. The data shown below were generated using the final version of the device, Gen.2.1.

*a. Precision/Reproducibility:*

Reaction mix inter-lot reproducibility was evaluated for Simplexa™ Flu A/B and RSV

Direct Gen 2.1 using three reaction mix lots. The sample panel used for testing consisted of one low positive each for influenza A, influenza B, and RSV, one medium positive each for influenza A, influenza B, and RSV, one negative and one positive control for a total of 8 samples in the panel. Low positive samples were created by spiking influenza A, influenza B, or RSV organisms into negative nasopharyngeal swab matrix at 1.5 times LoD. Medium positive samples were created by spiking influenza A, influenza B, or RSV organisms into negative nasopharyngeal swab matrix at 4 times LoD. Pooled negative nasopharyngeal swab matrix was used as the negative samples. Each sample panel member was tested in duplicate per reaction mix lot in each run, two runs per day for total of three days. The study was performed using a total of four Integrated Cyclers, each operated by a different operator. One lot of Simplexa™ Flu A/B & RSV Direct Positive Control Pack was used throughout testing. The Negative Template Control (NTC) and Positive Control were tested on each instrument once each day as a control set. The table below shows the strains and the concentrations of the sample panel.

Sample Panel Member	Target Virus	Strain (if spiked)	Concentration (TCID <sub>50</sub> /mL)
Flu A Low Positive	Flu A	Influenza A/Hong Kong/8/68 H3N2	0.15
Flu A Medium Positive			0.4
Flu B Low Positive	Flu B	Influenza B/Malaysia/2506/04	1.5
Flu B Medium Positive			4
RSV Low Positive	RSV	RSV-B CH93-18(18)	3
RSV Medium Positive			8
Negative	None	N/A	N/A
Positive Control (Lot # 25451)	All	N/A	N/A

The precision estimates were calculated using mixed effect nested model. The summary of the data is presented below.

Quantitative Summary of Reaction Mix Inter-Lot Reproducibility													
Analyte (Channel)	Sample Panel Member	N	Mean	Inter-Day		Inter-Run		Inter-Lot		Intra-Run/Lot		Total	
			Ct	SD	%C V	SD	%C V	SD	%C V	SD	%C V	SD	%C V
Flu A (FAM)	Flu A Low Positive	36	37	0	0	0.3	0.9	0	0	0.7	1.9	0.8	2.1
	Flu A Moderate Positive	36	35.2	0	0	0	0	0.4	1	0.4	1.1	0.5	1.5
	Positive Control (PC)	36	33.3	0.1	0.3	0.2	0.5	0.2	0.5	0.2	0.5	0.3	1
Flu B (JOE)	Flu B Low Positive	36	35.3	0	0	0	0	0.3	0.8	0.7	1.9	0.7	2.1

Quantitative Summary of Reaction Mix Inter-Lot Reproducibility													
Analyte (Channel)	Sample Panel Member	N	Mean	Inter-Day		Inter-Run		Inter-Lot		Intra-Run/Lot		Total	
			Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
	Flu B Moderate Positive	41*	33.5	0	0	0.1	0.4	0.3	0.9	0.3	1	0.5	1.4
	Positive Control (PC)	36	32.7	0	0	0	0	0.4	1.1	0.2	0.7	0.4	1.3
RSV (CFR610)	RSV Low Positive	36	36.5	0	0	0	0	0	0	0.8	2.3	0.8	2.3
	RSV Moderate Positive	36	34.6	0	0	0.2	0.6	0	0	0.5	1.4	0.5	1.5
	Positive Control (PC)	36	32.4	0.1	0.4	0.1	0.2	0	0.1	0.2	0.6	0.2	0.7
RNA IC (Q670)	Flu A Low Positive	36	29	0	0	0	0	0.4	1.5	0.4	1.2	0.6	1.9
	Flu A Moderate Positive	36	29.1	0	0	0	0	0.4	1.4	0.4	1.2	0.6	1.9
	Flu B Low Positive	36	28.2	0.2	0.5	0	0	0.2	0.8	0.3	1	0.4	1.4
	Flu B Moderate Positive	41*	28.2	0	0.1	0	0.1	0.2	0.7	0.2	0.7	0.3	1
	RSV Low Positive	36	28.9	0	0	0	0	0.2	0.8	0.3	1.1	0.4	1.3
	RSV Moderate Positive	36	28.9	0	0	0	0	0.3	0.9	0.3	0.9	0.4	1.2
	Negative	36	29.2	0	0	0.1	0.2	0	0	0.3	1	0.3	1
	Positive Control (PC)	36	28.9	0	0.1	0	0	0.2	0.7	0.1	0.4	0.2	0.8

\*The flu B moderate positive sample was tested in 41 replicates due to inadvertently testing flu B moderate positive instead of flu B low positive.

All samples generated expected results 100% of the time. The modified device demonstrated acceptable reproducibility with the total %CV values ranging from 1% to 2.3%.

See k120413 for additional information and for site-to-site reproducibility.

*b. Linearity/assay reportable range:*

Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

See k120413.

d. *Detection limit:*

Three strains of influenza A, two strains of influenza B and two strains of RSV were tested to determine the limit of detection (LoD) for the Simplexa™ Flu A/B & RSV Direct Gen 2.1 assay kit. Serial dilutions of quantified virus stocks spiked into negative nasopharyngeal swab matrix were prepared. Four concentrations per virus were tested in triplicate during the screening phase to determine the tentative LoD for each target virus. The lowest concentration with all three replicates of the target virus detected during the screening phase was tested in thirty two (32) replicates to confirm the reliable detection at that concentration. The LoD is determined to be the concentration at which at least 31/32 ( $\geq 95.0\%$ ) replicates are detected. The comparison of the limit of detection of the Simplexa Flu A/B & RSV between the original device (Gen 1.0) and the modified device (Gen 2.1) is shown below.

<b>Virus</b>	<b>Gen 1 (TCID<sub>50</sub>/mL)</b>	<b>Gen 2.1 (TCID<sub>50</sub>/mL)</b>
Influenza A/ Hong Kong/8/68 (H3N2)	10	0.1
Influenza A/PR/8/34 (H1N1)	0.005	0.05
Influenza A/Swine NY/02/2009 (H1N1)	0.1	1
Influenza B/Great Lakes/1739/54	2	20
Influenza B/Malaysia/2506/2004	20	1
RSV-A2	1	2
RSV B CH93-18 (18)	3	2

e. *Analytical specificity:*

Cross-reactivity

Thirty two (32) organisms were tested at clinically relevant concentrations to evaluate cross-reactivity of the Simplexa Flu A/B & RSV Direct Gen 2.1 Kit. Three instruments were used to perform 15 experimental runs across two days by a single operator. One lot of reaction mix and one lot of positive control were used to conduct the study. Each organism was spiked into negative nasopharyngeal swab matrix and tested in triplicate. A total of five replicates of baseline negative nasopharyngeal swab matrix were tested. One set of controls, a Negative Template Control (NTC) and a Positive Control, was tested on each instrument on each day of testing. No cross-reactivity was observed with the organisms tested, as shown below.

Organism	Tested Conc.	N	%Detection (# Detected / # Total)			
			Flu A (FAM)	Flu B (JOE)	RSV (CFR610)	IC (Q670)
Baseline	N/A	5	0.0%(0/5)	0.0%(0/5)	0.0%(0/5)	100.0%(5/5)
Adenovirus 1	4.17E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Adenovirus 7A	5.37E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Bordetella pertussis</i> A639	1.88E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Chlamydia pneumoniae</i>	1.00E+06 IFU/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Cytomegalovirus (CMV)	1.04E+05 U/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Coronavirus 229E	5.89E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Coronavirus OC43	1.95E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Corynebacterium diphtheriae</i>	4.00E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Enterovirus Type 71	1.10E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Epstein-Barr Virus (EBV)	1.10E+05 copies/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Escherichia coli</i> O157:H7	1.10E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Haemophilus influenzae</i>	1.41E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Lactobacillus plantarum</i> , 17-5	7.97E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Legionella longbeachae</i>	8.63E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Measles	1.95E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Metapneumovirus 9	1.58E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Moraxella catarrhalis</i> , NE1	1.49E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Mumps	8.51E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Mycobacterium tuberculosis</i> (genomic DNA)	6.54E+06 c/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Mycoplasma pneumoniae</i> , M129	3.16E+06 ccu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Neisseria elongata</i>	2.05E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Neisseria meningitidis</i>	7.07E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Parainfluenza 1	1.15E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Parainfluenza 2	3.80E+05 IU/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)

Organism	Tested Conc.	N	%Detection (# Detected / # Total)			
			Flu A (FAM)	Flu B (JOE)	RSV (CFR610)	IC (Q670)
Parainfluenza 3	1.95E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Pseudomonas aeruginosa</i>	3.93E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Rhinovirus 1A	1.26E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Staphylococcus aureus</i> , COL	1.42E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Staphylococcus epidermidis</i>	9.23E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Streptococcus pneumoniae</i>	9.20E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Streptococcus pyogenes</i> , M1	1.36E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
<i>Streptococcus salivarius</i>	2.12E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)

### Interference

Ten relevant potentially interfering substances were tested to evaluate their effect on the performance of the Simplexa™ Flu A/B & RSV Direct Gen 2.1 Kit. One baseline sample was prepared containing all three target viruses (influenza A, influenza B, and RSV) at the level of 3 times the determined LoD of each virus. Each of the potential interferents was spiked into a baseline sample and tested in triplicate. All dilutions were prepared in negative nasopharyngeal swab matrix. The virus strains and the concentrations used in the sample preparation are shown below.

Virus	LoD in TCID <sub>50</sub> /mL	3X LoD in TCID <sub>50</sub> /mL
Influenza A/Hong Kong/8/68 H3N2	0.1	0.3
Influenza B/Malaysia/2506/04	1.0	3.0
RSV-B CH93-18(18)	2.0	6

There was no interference observed at the concentrations tested, as shown below.

Substance	Concentration Tested	Qualitative Result for each Channel			
		Flu A (FAM)	Flu B (Joe)	RSV (CFR610)	RNA IC (Q670)
Baseline	None	100.0%(15/15)	100.0%(15/15)	100.0%(15/15)	100.0%(15/15)
Afrin Nasal Spray	15% (v/v)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Oseltamivir phosphate	1µM	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)

Substance	Concentration Tested	Qualitative Result for each Channel			
		Flu A (FAM)	Flu B (Joe)	RSV (CFR610)	RNA IC (Q670)
Blood	2% (v/v)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Zicam Nasal Gel	5% (v/v)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Tobramycin	4 µg/mL	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Mupirocin	6.6 mg/mL	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Purified Mucin Protein	60 µg/mL	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Beconase AQ	5% (v/v)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Nasal Corticosteroid - Fluticasone	5% (v/v)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
Relenza Antiviral Drug - Zanamivir	3.3 mg/mL	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)
NTC	NA	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Positive Control	NA	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)	100.0%(3/3)

*f. Analytical reactivity*

A total of 34 viruses (19 strains of influenza A (including H1, H3 and H5 subtypes), 11 strains of influenza B and 4 strains of RSV) were tested to evaluate analytical reactivity of the modified (Gen. 2.1) Simplexa Flu A/B & RSV Direct assay. The most recent strains and geographically diverse strains were chosen. A total of three 3M Integrated Cyclers were used to perform 19 experimental runs over four days by a single operator. One lot of reaction mix and one lot of Positive Control were used to conduct the study. Each organism was spiked into negative nasopharyngeal swab matrix and tested at least in triplicate. Total of five replicates of the baseline negative nasopharyngeal swab matrix were tested. One set of controls (NTC and Positive Control) was tested on each instrument on each day of testing. With the exception of the influenza A/Ohio/02/2012 (H3N2), all of the analytical reactivity panel viruses were detected with the modified device at the same concentrations as they were with the original device. The influenza A/Ohio/02/2012 (H3N2) was detected at 200 CEID<sub>50</sub>/mL by the modified device while the original device (Gen 1.0) detected the same strain at 100 CEID<sub>50</sub>. The newly tested influenza A/Anhui/1/2013 (H7N9) virus was detected at 25,000 EID<sub>50</sub>/mL.

Organism	Concentration Tested	Result
Influenza A/Taiwan/42/06 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Anhui/1/2013 (H7N9)	25,000 EID <sub>50</sub> /mL	Flu A Detected
Influenza A/Brisbane/10/07 H3	100 TCID <sub>50</sub> /mL	Flu A Detected

<b>Organism</b>	<b>Concentration Tested</b>	<b>Result</b>
Influenza A/Brisbane/59/07 H1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/California/12/2012 (H1N1) pdm09	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/California/7/2009 NYMC x-179-A	100 IU/mL	Flu A Detected
Influenza A/Indiana/08/2011 (H3N2)v	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Minnesota/11/2010 (H3N2)v	100 CEID <sub>50</sub> /mL	Flu A Detected
Influenza A/New Caledonia/20/99 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Ohio/02/2012 (H3N2)	200 CEID <sub>50</sub> /mL	Flu A Detected
Influenza A/Port Chalmers/1/73 H3N2	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/PR/8/34 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Solomon Island/03/06	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Swine NY/02/2009 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Swine/1976/31 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Swine/Iowa/15/30 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Texas/50/2012 (H3N2)	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/Wisconsin/67/05 H3	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza A/WS/33 H1N1	100 TCID <sub>50</sub> /mL	Flu A Detected
Influenza B/Allen/45	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Brisbane/60/2008	100 CEID <sub>50</sub> /mL	Flu B Detected
Influenza B/Florida/02/2006	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Florida/04/2006	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Florida/07/04	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Hong Kong/5/72	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Lee/40	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Maryland/1/59	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Panama/45/90	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Taiwan/2/62	100 TCID <sub>50</sub> /mL	Flu B Detected
Influenza B/Wisconsin/01/2010	100 CEID <sub>50</sub> /mL	Flu B Detected
RSV-A Long	100 TCID <sub>50</sub> /mL	RSV Detected

Organism	Concentration Tested	Result
RSV-B 9320	100 TCID <sub>50</sub> /mL	RSV Detected
RSV-B Wash/18537/62	100 TCID <sub>50</sub> /mL	RSV Detected
RSV B WV/14617/85	100 TCID <sub>50</sub> /mL	RSV Detected

NOTE: Although this test has been shown to detect novel avian influenza A (H7N9) and H3N2v cultured viruses, the performance characteristics of this device with clinical specimens that are positive for novel avian influenza A (H7N9) and H3N2v influenza viruses have not been established.

*g. Assay cut-off:*

Refer to k120413 for additional performance studies.

2. Comparison studies:

*a. Method comparison with predicate device:*

The modified device was validated with clinical samples in each of the two phases of the changes, Gen 2.0 and Gen 2.1.

1. Comparison between Simplexa™ Flu A/B & RSV Direct Gen 1.0 and Simplexa™ Flu A/B & RSV Direct Gen 2.0

The changes implemented for Gen 2.0 were evaluated with archived clinical samples. Based on historical data, 265 samples in Universal Transport Medium (UTM) or Viral Transport Medium (VTM) were assembled with 55 samples positive for influenza A, 55 samples positive for influenza B, 55 samples positive for RSV and 100 samples negative for all of the viruses tested. The 265 samples tested included 131 archived clinical samples originally tested in the clinical study conducted in support of the 510(k) submission (K120413). The other 134 samples included 33 archived samples from the 2010-2011 flu season and 101 samples from the 2013-2014 flu season. The samples were tested in parallel using the Simplexa™ Flu A/B&RSV Gen 1.0 kit and the Simplexa™ Flu A/B&RSV Gen 2.0 kit. The positive percent agreement (PPA) and the negative percent agreement (NPA) between the results obtained with the Simplexa™ Gen 1.0 and the Simplexa™ Gen 2.0, for each virus, are shown below:

**Comparison between Simplexa™ Flu A/B & RSV Direct Gen 1.0 and Simplexa™ Flu A/B & RSV Direct Gen 2.0**

Influenza A		Simplexa™ Flu A/B & RSV Direct Gen 1.0	
Simplexa™ Flu A/B & RSV Direct Gen 2.0		Detected	Not Detected
	Detected	58	9*
	Not Detected	0	198
	Total	58	207
	PPA = 100% (58/58) 95% CI: (93.0; 100) NPA = 95.7% (198/207) 95% CI: (91.9; 97.7)		

\*7/9 samples that were positive for influenza A by Gen 2.0 but negative by Gen 1.0 were positive for influenza A by another FDA cleared NAT.

Influenza B		Simplexa™ Flu A/B & RSV Direct Gen 1.0	
Simplexa™ Flu A/B & RSV Direct Gen 2.0		Detected	Not Detected
	Detected	54	9*
	Not Detected	1**	201
	Total	55	210
	PPA = 98.2% (54/55), 95% CI: (90.4; 99.7) NPA = 95.7% (201/210), 95% CI: (92.1; 97.7)		

\*5/9 samples that were positive for influenza B by Gen 2.0 but negative by Gen 1.0 were positive for influenza B by another FDA cleared NAT.

\*\* 1 sample that was negative for influenza B by Gen. 2.0 but positive by Gen 1.0 was negative for influenza B by another FDA cleared NAT

RSV		Simplexa™ Flu A/B & RSV Direct Gen 1.0	
Simplexa™ Flu A/B & RSV Direct Gen 2.0		Detected	Not Detected
	Detected	45	9*
	Not Detected	1**	210
	Total	46	219
	PPA = 97.8% (45/46), 95% CI: (88.7; 99.6) NPA = 95.9% (210/219), 95% CI: (92.4; 97.8)		

\*3/9 samples that were positive for RSV by Gen 2.0 but negative by Gen 1.0 were positive for RSV by another FDA cleared NAT.

\*\* 1 sample that was negative for RSV by Gen. 2.0 but positive by Gen 1.0 was negative for RSV by another FDA cleared NAT

2. Comparison between Simplexa™ Flu A/B & RSV Direct Gen 2.0 and Simplexa™ Flu A/B & RSV Direct Gen 2.1

The changes implemented for Gen 2.1 were evaluated with archived clinical samples. Based on historical data, 265 samples in Universal Transport Medium (UTM) or Viral Transport Medium (VTM) were assembled with 55 samples positive for influenza A, 55 samples positive for influenza B, 55 samples positive for RSV and 100 samples negative for all of the viruses tested. The 265 samples tested included 125 archived clinical samples originally tested in the clinical study conducted in support of the 510(k) submission (K120413). The other 140 samples included 48 archived samples from the 2009-2010 flu season, 9 samples from 2012-2013 and 83 samples from the 2013-2014 flu season. The samples were tested in parallel using the Simplexa™ Flu A/B & RSV Gen 2.0 kit and the Simplexa Flu A/B & RSV Gen 2.1 kit. The positive percent agreement (PPA) and the negative percent agreement (NPA) between the results obtained with the Simplexa Flu A/B & RSV Gen 2.0 and the Simplexa Flu A/B & RSV Gen 2.1, for each virus, are shown below:

**Comparison between Simplexa™ Flu A/B & RSV Direct Gen 2.0 and Simplexa™ Flu A/B & RSV Direct Gen 2.1**

Influenza A		Simplexa™ Flu A/B & RSV Direct Gen 2.0	
Simplexa™ Flu A/B & RSV Direct Gen 2.1		<b>Detected</b>	<b>Not Detected</b>
	<b>Detected</b>	58	2
	<b>Not Detected</b>	0	205
	<b>Total</b>	58	207
<b>PPA = 100.0% (58/58), 95% CI: (93.8; 100.0)</b> <b>NPA = 99.0% (205/207), 95% CI: (96.5; 99.7)</b>			

Influenza B		Simplexa™ Flu A/B & RSV Direct Gen 2.0	
Simplexa™ Flu A/B & RSV Direct Gen 2.1		<b>Detected</b>	<b>Not Detected</b>
	<b>Detected</b>	56	0
	<b>Not Detected</b>	0	209
	<b>Total</b>	56	209
<b>PPA = 100.0% (56/56), 95% CI: (93.6; 100.0)</b> <b>NPA = 100.0% (209/209), 95% CI: (98.2; 100.0)</b>			

RSV		Simplexa™ Flu A/B & RSV Direct Gen 2.0	
Simplexa™ Flu A/B & RSV Direct Gen 2.1		Detected	Not Detected
	Detected	55	0
	Not Detected	0	210
	Total	55	210
<b>PPA = 100.0% (55/55), 95% CI: (93.5; 100.0)</b> <b>NPA = 100.0% (210/210), 95% CI: (98.2; 100.0)</b>			

*b. Matrix comparison:*

Not applicable

3. Clinical studies:

Clinical performance characteristics of the Simplexa™ Flu A/B & RSV Direct assay were established in a clinical study with samples collected during the 2010/2011 influenza season when 2009 H1N1 and H3N2 were the predominant influenza A viruses in circulation. Please refer to k120413 for additional information.

*a. Clinical Sensitivity:*

Refer to k120413 for clinical performance studies.

*b. Clinical specificity:*

Refer to k120413 for clinical performance studies.

*c. Other clinical supportive data (when a. and b. are not applicable):*

4. Clinical cut-off:

See k120413

5. Expected values/Reference range:

See k120413

**N. Instrument Name:**

3M Integrated Cycler

**O. System Descriptions:**

See k120413

**P. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**Q. Conclusion:**

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