DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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

Focus Diagnostics, Inc.
Sharon Young,
Senior Regulatory Affairs Specialist
11331 Valley View Street
Cypress, CA 90630

December 5, 2014

Re: K142365
Trade/Device Name: Simplexa™ Flu A/B & RSV Direct
Simplexa™ Flu A/B & RSV Positive Control Pack
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: Class II
Product Code: OCC, OOI
Dated: September 26, 2014
Received: September 29, 2014

Dear Ms. Young:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set

forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K142365

Device Name
Simplexa™ Flu A/B & RSV Direct and Simplexa™ Flu A/B & RSV Positive Control Pack

Indications for Use *(Describe)*

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Applicant	Focus Diagnostics, Inc. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Sharon Young tel 562.240.6680 fax 562.240.6529 syoung@focusdx.com
Summary Date	December 2, 2014
Proprietary Name	Simplexa™ Flu A/B & RSV Direct and Simplexa™ Flu A/B & RSV Positive Control Pack
Generic Name	Respiratory viral panel nucleic acid
Classification	Class II
US Product Code	OCC - Respiratory Viral Panel Nucleic Acid Assay System OOI - Real Time Nucleic Acid Amplification System
Regulation Number	21 CFR § 866.3980
Predicate Device	K120413 Simplexa™ Flu A/B & RSV Direct and Simplexa™ Flu A/B & RSV Positive Control Pack

510(k) SUBMISSION PURPOSE

The purpose of the submission is to add eight (8) additional influenza strains to the Analytical Reactivity of the Simplexa™ Flu A/B & RSV Direct. The package insert was updated to include the additional strains which included the following six (6) additional strains of Influenza A and two (2) additional strains of Influenza B:Influenza A/Anhui/1/2013, 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.

Changes to the Simplexa™ Flu A/B and RSV Direct K120413 (Gen 1) 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.

INTENDED USE

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.

DEVICE DESCRIPTION

The Simplexa™ Flu A/B & 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 & RSV Direct assay, the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct 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. The assay provides three results; conserved regions of influenza A viruses (matrix gene), influenza B viruses (matrix gene) and RSV (M gene) are targeted to identify these viruses in the specimen. An RNA internal control is used to detect RT-PCR failure and/or inhibition.

MATERIALS PROVIDED

The Focus Diagnostics Simplexa™ Flu A/B & RSV Direct kit contains sufficient reagents for 24 reactions. Upon receipt, store at -10 to -30 ºC (do not use a frost-free freezer). Each vial contains sufficient material for one use. Use within 30 minutes of removing from the freezer.

Kit Description

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ Flu A/B & RSV Direct Reaction Mix	**MOL2651**	**REAG**	**A**	**RM**	Brown	24	1/24	50 µL

Component Description

Kit Component	Contents				
Simplexa™ Flu A/B & RSV Direct Reaction Mix (RM)	DNA polymerase, Reverse Transcriptase, RNase inhibitor, buffer and dNTPs, encapsulated RNA Template Dye-labeled fluorescent primers specific for detection of influenza A, influenza B and RSV and for the Internal Control				
	Target	**Probe Fluorophore (Dye)**	**Excitation**	**Emission**	**Targeted Gene**
	Flu A	FAM	495	520	matrix
	Flu B	JOE	520	548	matrix
	RSV	CFR610	590	610	M gene
	Internal Control "RNA IC"	Q670	644	670	N/A
Simplexa™ Flu A/B & RSV Direct Barcode Card	Assay specific parameters, lot number, expiration date				

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

Product Description

Component Name	REF	Description	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ Flu A/B & RSV Direct Positive Control	**MOL2661**	Inactivated influenza A virus, Inactivated influenza B virus, Inactivated RSV	Red	10	1/10	100 µL

MATERIALS SUPPLIED SEPARATELY

1. Direct Amplification Disc Kit (REF MOL1455)
    a. Direct Amplification Discs for use on the Integrated Cycler.

INSTRUMENT REQUIREMENTS

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

COMPARISON TO PREDICATE

Similarities

Feature	Predicate K120413	Modified Device K142365
Intended Use	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	Same

Feature	Predicate K120413	Modified Device K142365
	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.	
Technology	The Simplexa™ Flu A/B & 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 & RSV Direct assay, the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct 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. The assay provides three results; conserved regions of influenza A viruses (matrix gene), influenza B viruses (matrix gene) and RSV (M gene) are targeted to identify these viruses in the specimen. An RNA internal control is used to detect RT-PCR failure and/or inhibition.	Same
Instrument	3M Integrated Cycler	Same
Specimen Type	Unprocessed nasopharyngeal swabs that have not undergone nucleic acid extraction	Same
Influenza A Viral Target	Well conserved region of the matrix gene	Same
Influenza B Viral Target	Well conserved region of the matrix gene	Same
Respiratory Syncytial Viral Target	M gene	Same
Assay Type	Qualitative	Same

Differences

	Predicate K120413	Modified Device K142365
Analytical Reactivity	Influenza A Brisbane/10/07 H3, Influenza A Brisbane/59/07 H1, Influenza A New Caledonia/20/99 H1N1, Influenza A Port Chalmers/1/73 H3N2, Influenza A Solomon Island/03/06 H1, Influenza A Taiwan/42/06 H1N1, Influenza A Wisconsin/67/05 H3, Influenza A WS/33 H1N1, Influenza A/California/7/2009 NYMC x-179-A , Tissue Culture Adapted Influenza A/Swine H1N1/Iowa/15/1930, Tissue Culture Adapted Influenza A Swine H1N1/USA/1976/1931, Influenza A PR8 Vietnam/1203/2004 (H5N1 - inactivated virus), Influenza B Allen/45, Influenza B Florida/02/2006, Influenza B Florida/04/2006, Influenza B Florida/07/04, Influenza B Hong Kong/5/72, Influenza B Lee/40, Influenza B Maryland/1/59, Influenza B Panama/45/90, and Influenza B Taiwan/2/62,	Eight (8) additional influenza strains were tested and included in the Analytical Reactivity table. The added strains were 6 strains of Influenza A and 2 additional strains of Influenza B; Influenza A/Anhui/1/2013, Influenza A/California/12/2012 (H1N1) pdm09, Influenza A/Indiana/08/2011 (H3N2)v, Influenza A/Minnesota/11/2010 (H3N2)v, Influenza, Influenza A/Ohio/02/2012 (H3N2), Influenza A/Texas/50/2012 (H3N2), Influenza B/Brisbane/60/2008 and Influenza B/Wisconsin/01/2010 List including the new strains; Influenza A Brisbane/10/07 H3, influenza A Brisbane/59/07 H1, influenza A New Caledonia/20/99 H1N1, influenza A Port Chalmers/1/73 H3N2, influenza A Solomon Island/03/06 H1, iinfluenza A Taiwan/42/06 H1N1, influenza A Wisconsin/67/05 H3, influenza A WS/33 H1N1, influenza A/California/7/2009 NYMC x-179-A, Tissue Culture Adapted influenza A/Swine H1N1/Iowa/15/1930, Tissue Culture Adapted iinfluenza A Swine H1N1/USA/1976/1931, influenza A PR8 Vietnam/1203/2004 (H5N1 - inactivated virus), influenza A/Anhui/1/2013 (H7N9), 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 Allen/45, influenza B Florida/02/2006, influenza B Florida/04/2006, influenza B Florida/07/04, influenza B Hong Kong/5/72, influenza B Lee/40, influenza B Maryland/1/59, influenza B Panama/45/90, influenza B Taiwan/2/62, influenza B/Brisbane/60/2008, influenza B/Wisconsin/01/2010, RSV A Long, RSV B 9320, RSV B Wash/18537/62, RSV B WV/14617/85.

	Predicate K120413	Modified Device K142365
Manufacturing, Material and Cycling Parameters		(Gen 2.0) - Changes to the annealing temperature (decreased), change in the run time (increased) and changes in enzyme used and change to the RSV cut-off Ct (decreased). (Gen 2.1) - Changes to manufacturing process and materials.

Method Comparison - Simplexa™ Flu A/B & RSV Direct Gen 1.0 (K120413) to Simplexa™ Flu A/B & RSV Direct Gen 2.0

The changes implemented for Simplexa™ Flu A/B & RSV Direct (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 Direct (Gen 1.0) and the Simplexa™ Flu A/B & RSV Direct (Gen 2.0). The positive percent agreement (PPA) and the negative percent agreement (NPA) between the results obtained with the Simplexa™ Flu A/B & RSV Direct (Gen 1.0) and the Simplexa™ Flu A/B & RSV Direct (Gen 2.0) for each virus are shown below:

Method Comparison - Simplexa™ Flu A/B & RSV Direct Gen 2.0 to Simplexa™ Flu A/B & RSV Direct Gen 2.1(K142365)

The changes implemented for Simplexa™ Flu A/B & RSV Direct (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 2010-2011 flu season, 9 samples from 2012-2013 flu season and 83 samples from the 2013-2014 flu season. The samples were tested in parallel using the Simplexa™ Flu A/B & RSV Direct (Gen 2.0) and the Simplexa™ Flu A/B & RSV Direct (Gen 2.1). The positive percent agreement (PPA) and the negative percent agreement (NPA) between the results obtained with the Simplexa™ Flu A/B & RSV Direct (Gen 2.0) and the Simplexa™ Flu A/B & RSV Direct (Gen 2.1) for each virus are shown below:

Influenza A - Simplexa™ Flu A/B & RSV Direct Gen 1.0 (K120413) to Simplexa™ Flu A/B & RSV Direct Gen 2.0

Simplexa™ Flu A/B & RSV Direct (Gen 2.0) Flu A Result	Simplexa™ Flu A/B & RSV Direct (Gen 1.0) Flu A Result		Grand Total		%Agreement	95% CI
	Detected	Not Detected				
Detected	58	9[a]	67	PPA	100.0% (58/58)	93.0% to 100.0%
Not Detected	0	198	198	NPA	95.7% (198/207)	91.9% to 97.7%
Grand Total	58	207	265			

[a]7/9 discrepant (K120413 – Negative and K142365 – Positive) samples were positive for Flu A on another FDA cleared NAT.

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement

Influenza A - Simplexa™ Flu A/B & RSV Direct Gen 2.0 to Simplexa™ Flu A/B & RSV Direct Gen 2.1 (K142365)

Simplexa™ Flu A/B & RSV Direct (Gen 2.1) Flu A Result	Simplexa™ Flu A/B & RSV Direct (Gen 2.0) Flu A Result		Grand Total		%Agreement	95% CI
	Detected	Not Detected				
Detected	58	2	60	PPA	100.0% (58/58)	93.8% to 100.0%
Not Detected	0	205	205	NPA	99.0% (205/207)	96.5% to 99.7%
Grand Total	58	207	265			

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement

Influenza B - Simplexa™Flu A/B & RSV Direct Gen 1.0 (K120413) to Simplexa™ Flu A/B & RSV Direct Gen 2.0

Simplexa™ Flu A/B & RSV Direct (Gen 2.0) Flu B Result	Simplexa™ Flu A/B & RSV Direct (Gen 1.0) Flu B Result		Grand Total		%Agreement	95% CI
	Detected	Not Detected				
Detected	54	9[b]	63	PPA	98.2% (54/55)	90.4% to 99.7%
Not Detected	1[c]	201	202	NPA	95.7% (201/210)	92.1% to 97.7%
Grand Total	55	210	265			

[b]5/9 discrepant (K120413 – Negative and K142365 – Positive) samples were positive for Flu B on another FDA cleared NAT.

[c]1/1 discrepant (K120413 – Positive and K142365 – Negative) samples was negative for Flu B on another FDA cleared NAT.

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement

Influenza B - Simplexa™ Flu A/B & RSV Direct Gen 2.0 to Simplexa™ Flu A/B & RSV Direct Gen 2.1 (K142365)

Simplexa™ Flu A/B & RSV Direct (Gen 2.1) Flu B Result	Simplexa™ Flu A/B & RSV Direct (Gen 2.0) Flu B Result		Grand Total		%Agreement	95% CI
	Detected	Not Detected				
Detected	56	0	63	PPA	100.0% (56/56)	93.6% to 100.0%
Not Detected	0	209	202	NPA	100.0% (209/209)	98.2% to 100.0%
Grand Total	56	209	265			

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement

RSV - Simplexa™ Flu A/B & RSV Direct Gen 1.0 (K120413) to Simplexa™ Flu A/B & RSV Direct Gen 2.0

Simplexa™ Flu A/B & RSV Direct (Gen 2.0) RSV Result	Simplexa™ Flu A/B & RSV Direct (Gen 1.0) RSV Result		Grand Total		%Agreement	95% CI
	Detected	Not Detected				
Detected	45	9[d]	54	PPA	97.8% (45/46)	88.7% to 99.6%
Not Detected	1[e]	210	211	NPA	95.9% (210/219)	92.4% to 97.8%
Grand Total	46	219	265			

[d]3/9 discrepant (K120413 – Negative and K142365 – Positive) samples were positive for RSV on another FDA cleared NAT.

[e]1/1 discrepant (K120413 – Positive and K142365 – Negative) sample was negative for RSV on another FDA cleared NAT.

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement

RSV - Simplexa™ Flu A/B & RSV Direct Gen 2.0 to Simplexa™ Flu A/B & RSV Direct Gen 2.1 (K142365)

Simplexa™ Flu A/B & RSV Direct (Gen 2.1) RSV Result	Simplexa™ Flu A/B & RSV Direct (Gen 2.0) RSV Result		Grand Total		%Agreement	95% CI
	Detected	Not Detected				
Detected	55	0	55	PPA	100.0% (55/55)	93.5% to 100.0%
Not Detected	0	210	210	NPA	100.0% (210/210)	98.2% to 100.0%
Grand Total	55	210	265			

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement

ANALYTICAL REACTIVITY/ CROSS REACTIVITY

Analytical Reactivity - Simplexa™ Flu A/B & RSV Direct (Gen 2.1)

Different strains of influenza A including H1, H3 and H5 subtypes, influenza B and RSV including A and B subtypes were evaluated. The most recent strains and geographically diverse strains were chosen. Quantified viral material was spiked into negative swab matrix at a single dilution at the concentrations below. Each was assayed in triplicate. Ct values obtained during testing indicate all viral strains were tested near the LoD. All strains tested were appropriately detected.

Organism	Concentration Tested	Result
Influenza A/Taiwan/42/06 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Anhui/1/2013 (H7N9)	25,000 EID_{50}/mL	Flu A Detected
Influenza A/Brisbane/10/07 H3	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Brisbane/59/07 H1	100 $TCID_{50}$/mL	Flu A Detected

Organism	Concentration Tested	Result
Influenza A/California/12/2012 (H1N1) pdm09	100 $TCID_{50}$/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 $TCID_{50}$/mL	Flu A Detected
Influenza A/Minnesota/11/2010 (H3N2)v	100 $CEID_{50}$/mL	Flu A Detected
Influenza A/New Caledonia/20/99 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Ohio/02/2012 (H3N2)	200 $CEID_{50}$/mL	Flu A Detected
Influenza A/Port Chalmers/1/73 H3N2	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/PR/8/34 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Solomon Island/03/06	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Swine NY/02/2009 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Swine/1976/31 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Swine/Iowa/15/30 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Texas/50/2012 (H3N2)	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/Wisconsin/67/05 H3	100 $TCID_{50}$/mL	Flu A Detected
Influenza A/WS/33 H1N1	100 $TCID_{50}$/mL	Flu A Detected
Influenza B/Allen/45	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Brisbane/60/2008	100 $CEID_{50}$/mL	Flu B Detected
Influenza B/Florida/02/2006	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Florida/04/2006	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Florida/07/04	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Hong Kong/5/72	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Lee/40	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Maryland/1/59	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Panama/45/90	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Taiwan/2/62	100 $TCID_{50}$/mL	Flu B Detected
Influenza B/Wisconsin/01/2010	100 $CEID_{50}$/mL	Flu B Detected
RSV-A Long	100 $TCID_{50}$/mL	RSV Detected
RSV-B 9320	100 $TCID_{50}$/mL	RSV Detected

Organism	Concentration Tested	Result
RSV-B Wash/18537/62	100 $TCID_{50}$/mL	RSV Detected
RSV B WV/14617/85	100 $TCID_{50}$/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.

Cross Reactivity (Analytical Specificity) - Simplexa™ Flu A/B & RSV Direct (Gen 2.1)

Thirty-two (32) organisms were tested at clinically relevant concentrations to evaluate cross-reactivity of the Simplexa™ Flu A/B & RSV Direct. Three (3) instruments were used and fifteen (15) experimental runs across two (2) days by a single (1) operator were performed. Each of the organisms was spiked into a negative matrix and tested in triplicate (3). Baseline negative matrix was tested in five (5) replicates. No cross reactivity was observed.

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)
Bordetella pertussis A639	1.88E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Chlamydia pneumoniae	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)
Corynebacterium diphtheriae	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)
Escherichia coli O157:H7	1.10E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Haemophilus influenzae	1.41E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Lactobacillus plantarum, 17-5	7.97E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Legionella longbeachae	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)
Moraxella catarrhalis, NE1	1.49E+06 cfu/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)
Mumps	8.51E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Mycobacterium tuberculosis (genomic DNA)	6.54E+06 c/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Mycoplasma pneumoniae, M129	3.16E+06 ccu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Neisseria elongata	2.05E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Neisseria meningitides	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)
Parainfluenza 3	1.95E+05 TCID50/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Pseudomonas aeruginosa	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)
Staphylococcus aureus, COL	1.42E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Staphylococcus epidermidis	9.23E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Streptococcus pneumoniae	9.20E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Streptococcus pyogenes, M1	1.36E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)
Streptococcus salivarius	2.12E+06 cfu/mL	3	0.0%(0/3)	0.0%(0/3)	0.0%(0/3)	100.0%(3/3)

INTERFERENCE - Simplexa™ Flu A/B & RSV Direct (Gen 2.1)

The performance of this assay was evaluated with potentially interfering substances that may be present in nasopharyngeal swabs at the concentrations indicated in the table below. The potentially interfering substances were evaluated in a contrived sample that contained influenza A, influenza B and RSV. All strains were tested at two to four times the LoD. There was no evidence of interference caused by the substances tested.

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)
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)

Substance	Concentration Tested	Qualitative Result for each Channel			
		Flu A (FAM)	Flu B (Joe)	RSV (CFR610)	RNA IC (Q670)
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)

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

Three (3) strains of influenza A, two (2) strains of influenza B and two (2) strains of RSV were tested to determine the limit of detection (LoD) for the Simplexa™ Flu A/B & RSV Direct. Four concentrations per virus were tested in triplicate during the screening phase to determine the tentative LoD. The lowest concentration with all replicates detected during the screening phase was tested in thirty two (32) replicates to confirm detection at that concentration. The LoD is determined if at least 31/32 (≥95.0%) replicates are detected. The following table shows the comparison of the limit of detection between the original Simplexa™ Flu A/B & RSV Direct (Gen 1.0) and the modified Simplexa™ Flu A/B & RSV Direct (Gen 2.0 and Gen 2.1).

Virus	Gen 1.0 ($TCID_{50}$/mL)	Gen 2.0 ($TCID_{50}$/mL)	Gen 2.1 ($TCID_{50}$/mL)
Influenza A/ Hong Kong/8/68 (H3N2)	10	0.1	0.1
Influenza A/PR/8/34 (H1N1)	0.005	0.05	0.05
Influenza A/Swine NY/02/2009 (H1N1)	0.1	1	1
Influenza B/Great Lakes/1739/54	2	20	20
Influenza B/Malaysia/2506/2004	20	0.5	1
RSV-A2	1	2	2
RSV B CH93-18 (18)	3	2	2

PRECISION - Simplexa™ Flu A/B & RSV Direct (Gen 2.1)

The reaction mix (RM) lot to lot reproducibility was evaluated for Simplexa™ Flu A/B & RSV Direct (Gen 2.1) using three (3) Reaction Mix lots and sample panel which includes Low (~1.5 X LoD) and Moderate (~4 X LoD) positive samples for each of the three targets (Flu A, Flu B and RSV) and Positive Control. The low and moderate positive samples of each target were prepared by spiking the viral strain into negative swab matrix.

Each sample panel member was tested in duplicate (2) for each Reaction Mix lot in each run, two (2) runs per day for total of three (3) days using a single (1) 3M Integrated Cycler and a single (1) operator. The study produced at least thirty-six (36) replicates for each sample panel member. The Flu B moderate positive sample was tested in forty-one (41) replicates.

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 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
	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	36	28.9	0	0.1	0	0	0.2	0.7	0.1	0.4	0.2	0.8

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**
	(PC)												

Qualitative Results (By Sample)	**All**	**Flu A (FAM)**				**Flu B (JOE)**				**RSV (CFR610)**			
		Detected		**Not Detected**		**Detected**		**Not Detected**		**Detected**		**Not Detected**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Flu A Low Positive	36	36	100.0 %					36	100.0 %			36	100.0 %
Flu A Medium Positive	36	36	100.0 %					36	100.0 %			36	100.0 %
Flu B Low Positive	36			36	100.0 %	36	100.0 %					36	100.0 %
Flu B Medium Positive	41			41	100.0 %	41	100.0 %					41	100.0 %
RSV: Low Positive	36			36	100.0 %			36	100.0 %	36	100.0 %		
RSV: Medium Positive	36			36	100.0 %			36	100.0 %	36	100.0 %		
Negative Template Control (NTC)	36			36	100.0 %			36	100.0 %			36	100.0 %
Positive Control (PC)	36	36	100.0 %			36	100.0 %			36	100.0 %		

ADDITIONAL PERFORMANCE STUDIES

Please refer to the previously FDA cleared 510(k) K120413 for additional information.