510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k120413

B. Purpose for Submission:

This is a new 510(k) application for a qualitative Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay used with the 3M Integrated Cycler instrument for the *in vitro* qualitative detection and discrimination of influenza A virus, influenza B virus and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from symptomatic human patients.

C. Measurand:

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:

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay for the qualitative detection and differentiation of influenza A virus, influenza B virus and RSV RNA in nasopharyngeal swabs. The assay measures viral RNA directly from unextracted 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 Cycler with Integrated Cycler 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
   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 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
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:

   To be used with the 3M™ Integrated Cycler with Integrated Cycler Studio Software version 4.2 or higher

I. Device Description:

The test is a real-time RT-PCR amplification and detection assay 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 RNA in nasopharyngeal swabs. The assay uses direct measurement of viral RNA without extraction. 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 (IC). 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 inhibition.

The instrument utilizes a consumable named the Direct Amplification Disc (DAD) to process specimens. The DAD consumable is compartmentalized into eight separate wedges, up to eight separate specimens or controls may be processed on each disc. Each wedge contains sample and reagent input ports, microfluidic channels and laser activated valves to control the fluid flow and a reaction chamber. This disc meters the amount of reagent (reaction mix) and sample that are placed into specific ports in the disc. A foil seal is lifted and the user adds 50 µL of reaction mix to the reagent input port using a fixed volume pipette. Centrifugal force moves the fluid into the metering chamber. The reagent chamber measures 40 µL of reagent and the sample chamber measures 10 µL of sample. Excess reagent and sample are forced by centrifugal forces into the waste chambers.

The Simplexa™ Flu A/B & RSV Direct kit contains the reaction mix (RM), the Simplexa™ Flu A/B & RSV Direct Barcode Card with assay specific information, and Package Insert.

Simplexa™ Flu A/B & RSV Direct reaction mix contents:

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

Materials supplied separately: Direct Amplification Discs for use on the Integrated Cycler

Materials required but not supplied: 3M Integrated Cycler with Integrated Cycler Studio Software version 4.2 or higher, Simplexa™ Flu A/B & RSV Positive Control Pack, 50 µL fixed volume pipette (VWR Signature™ Fixed Volume Ergonomic High-Performance Pipettor Model VWR FE50 or equivalent), Sterile nuclease-free disposable pipette tips with filters, freezer (manual defrost) at -10 to -30 °C (for kit component and specimen frozen storage), refrigerator at 2 to 8 °C (for specimens).

Recommended materials: Universal Transport Media (UTM) to be used as a No Template Control (NTC), Replacement Foil Wedges

Assay Procedure:

1. Select samples that need to be tested.
2. Thaw reaction-mix vials at room temperature (approximate range 18 to 25 °C). Thaw one reaction-mix vial for each sample or control to be tested.
3. Scan the barcode on the Simplexa™ Flu A/B & RSV Direct Reaction Mix vial or barcode card.
4. Scan the disc barcode on the Direct Amplification Disc (DAD).
5. Scan or type in each sample identifier.
6. For one wedge at a time, peel the adhesive foil back to expose the Sample (SAMPLE) and Reaction (R) wells without completely removing the adhesive foil cover. Avoid touching the underside of the foil that will be in contact with the wells and disc surface.
7. Ensure that the reaction mix is completely thawed. Briefly spin down the tubes as needed. (Do not vortex the reaction mix)
8. Use the fixed volume pipette to transfer 50 µL of the reaction-mix into Reaction (R) well.
9. Use the fixed volume pipette to transfer 50 µL of samples or control; pipette sample or control into Sample well (SAMPLE).

10. Cover the wedge sealing the wells with the peeled adhesive foil, pressing down firmly near the edge of the wedge. If the original foil is torn it should be replaced with an extra replacement foil wedge.
11. Tear off the tab portion of the foil cover along the perforation.
12. Repeat steps 6 to 11 for the next sample(s).
13. Load the sealed Direct Amplification Disc into the Integrated Cycler and start the run.

Interpretation of Results:

The Integrated Cycler Studio software performs all of the results interpretation automatically. The display presents the user with a separate report box for each of the three analytes as well as the IC. The IC can be reported as "valid" or "invalid". Detection of the IC in test specimens is not required for a valid result. The software reports one of four possible outcomes after a run is completed for each sample ID:

Detected: Flu A, Flu B, or RSV was detected in the sample
Not Detected: Flu A, Flu B, or RSV was not detected in the sample
Invalid: An error occurred and the instrument could not conclusively detect Flu A, Flu B, RSV
EC500: The software detected a data quality error

An "Invalid" result can occur from RNA Internal Control failure or failure to detect sufficient specimen. Specimens should be retested in the event of an Invalid result. An "EC500" result can occur if the software was unable to determine a valid amplification curve for the analyte. Specimens should be retested in the event of an EC500 result.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Prodesse ProFlu™+

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

   k092500, k081030, k073029

3. Comparison with predicate:

Similarities		
Item	Simplexa™ Flu A/B & RSV Direct	Prodesse ProFlu™+
Intended Use	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 discrimination of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs 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 Virus were established using samples obtained when 2009 H1N1 influenza was the predominant Influenza A virus in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel	The Prodesse ProFlu™+ Assay is a multiplex Real-Time PCR in vitro diagnostic test for the rapid and qualitative detection and discrimination of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) nucleic acids isolated and purified from nasopharyngeal swab specimens obtained from symptomatic patients. This test is intended for use to 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 or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. It is recommended that negative RSV results be confirmed by culture. Performance characteristics for influenza A virus were established when influenza A/H3 and A/H1 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

Similarities		
Item	Simplexa™ Flu A/B & RSV Direct	Prodesse ProFlu™+
	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 in these cases unless a BSL3+ facility is available to receive and culture specimens.	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 in these cases unless a BSL3+ facility is available to receive and culture specimens.
510(k)	k120413	k073029, k081030, k092500
Regulation	21 CFR 866.3980	21 CFR 866.3980
Product Code	OCC	OCC
Assay Targets	RNA from Influenza A, Influenza B, and RSV	RNA from Influenza A, Influenza B, and RSV
Sample Types	Nasopharyngeal swabs	Nasopharyngeal swabs
Assay Type	Real-Time RT-PCR	Real-Time RT-PCR
Assay Results	Qualitative	Qualitative
Detection	Different fluorescent reporter dyes for each target	Different fluorescent reporter dyes for each target
Multiplex Capability for Influenza A, Influenza B, RSV	Yes	Yes
Influenza A target	Matrix gene	Matrix gene

Differences		
Item	Simplexa™ Flu A/B & RSV Direct	Prodesse ProFlu™+
Extraction Method	No extraction	Roche MagNA Pure LC System, bioMérieux NucliSENS easyMag
Assay Instrument	3M™ Integrated Cycler	Cepheid SmartCycler II System
Influenza B target	Matrix gene	Non-structural NS1 and NS2 genes
RSV target	M gene	Polymerase gene

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

Guidance for Industry and FDA Staff, Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay, October 9, 2009

Guidance for Industry and FDA Staff - Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses, July 15, 2011

Guidance for Industry and FDA Staff, In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path, May 1, 2007

Guidance for Industry and FDA Staff: Administrative Procedures for CLIA Categorization, May 7, 2008

Guidance for Industry and FDA Staff, Format for Traditional and Abbreviated 510(k), August 12, 2005

L. Test Principle:

The Simplexa™ Flu A/B & RSV assay is a nucleic acid amplification assay that uses Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) amplification to enable simultaneous and distinct detection of viral RNA from influenza A, influenza B and RSV from nasopharyngeal swabs from human patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.

The assay combines real-time PCR amplification with fluorescent signal detection technology. A bi-functional fluorescent probe-primer is used together with a reverse primer to amplify a specific target (for each analyte and internal control). A fluorescent signal is generated after separation of the fluorophore from the quencher as a result of the binding of a probe element to the extended DNA fragment synthesized during amplification.

The 3M Integrated Cycler is a rapid real-time PCR thermocycler used for the identification of nucleic acid from biological specimens. The instrument utilizes one of two types of disc media to contain and to process specimens: the Universal Disc and the Direct Amplification Disc (DAD). The instrument uses real time fluorometric detection to identify targets within the sample wells. The instrument is controlled by an external computer running the Integrated Cycler Studio Software.

The Simplexa™ Flu A/B & RSV Direct assay uses the DAD consumable. The DAD consumable is compartmentalized into eight separate wedges, up to separate specimens or controls may be processed on each disc. Each wedge contains sample and reagent input ports, microfluidic channels and laser activated valves to control the fluid flow and a reaction chamber. This disc is specifically designed to meter the amount of reagent (reaction mix) and

sample that are placed into specific ports in the disc. A foil seal is lifted and the user adds reaction mix to the reagent input port using a fixed volume pipette. Before sealing the well the user adds unextracted specimen to the sample input port.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility study evaluated the device's inter-laboratory, inter-assay, and intra-assay reproducibility for high negative, low positive and moderately positive specimens for influenza A, influenza B, and RSV. The testing panel consisted of high negative pool for all three targets, a low (approximately 2-4 times LoD) and medium positive (approximately 20 times LoD) for each analyte. These specimens were generated by spiking viral stock dilutions into pooled nasopharyngeal swab clinical matrix that was screened to be negative for all three analytes. Each sample was tested in triplicate at three separate sites. One run consisting of set of two discs, per operator, per day, two operators per site, were tested at three sites for five days. Three sites assessed the device's inter-laboratory reproducibility and inter/intra-assay reproducibility. Combined results for all sites and results stratified by site are presented in the tables below.

Reproducibility – Flu A

	Site 1			Site 2			Site 3			Overall	
Sample	Agreement with expected results	Mean Ct*	%CV	Agreement with expected results	Mean Ct*	%CV	Agreement with expected results	Mean Ct*	%CV	Total Agreement with expected results	95% CI
Flu A Low Positive	100% (30/30)	34.3	0.9	100% (30/30)	34.4	0.9	100% (30/30)	34.6	1.1	100% (90/90)	95.9-100%
Flu A Medium Positive	100% (30/30)	31.9	0.6	100% (30/30)	32.1	0.4	100% (30/30)	32.3	1.5	100% (90/90)	95.9-100%
Flu B Low Positive	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (90/90)	95.9-100%
Flu B Medium Positive	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (90/90)	95.9-100%
RSV Low Positive	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (90/90)	95.9-100%
RSV Medium Positive	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (30/30)	40.1	0	100% (90/90)	95.9-100%
High Negative	100% (30/30)	40.1	0	90% (27/30)	39.7	3	100% (30/30)	40.1	0	96.7% (87/90)	90.7-98.9%

Total Agreement	100% (210/210)	98.6% (207/210)	100% (210/210)	99.5% (627/630)	98.6-99.8%

Note: Samples that are negative for an analyte are assigned a value of 40.1 for the purposes of statistical analysis only. Samples that are negative have a Ct value of zero (0).

*Mean Ct for Flu A detection channel

Reproducibility – Flu B

Sample	Site 1			Site 2			Site 3			Overall	
	Agreement with expected results	Mean Ct*	%CV	Agreement with expected results	Mean Ct*	%CV	Agreement with expected results	Mean Ct*	%CV	Total Agreement with expected results	95% CI
Flu A Low Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
Flu A Medium Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
Flu B Low Positive	100% (30/30)	33.4	0.9	96.7% (29/30)	33.9	3.6	100% (30/30)	33.6	2.3	98.9% (89/90)	94-99.8%
Flu B Medium Positive	100% (30/30)	31.1	0.5	100% (30/30)	31.7	0.7	100% (30/30)	31.2	0.9	100% (90/90)	95.9-100%
RSV Low Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
RSV Medium Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
High Negative	96.7% (29/30)	40.0	1.0	93.3% (28/30)	39.9	2.4	96.7% (29/30)	40.1	0.0	95.6% (86/90)	89.1-98.3%
Total Agreement	99.5% (209/210)			98.6% (207/210)			99.5% (209/210)			99.4% (626/630)	98.2-99.7%

Note: Samples that are negative for an analyte are assigned a value of 40.1 for the purposes of statistical analysis only. Samples that are negative have a Ct value of zero (0).

*Mean Ct for Flu B detection channel

Reproducibility – RSV

Sample	Site 1			Site 2			Site 3			Overall	
	Agreement with expected results	Mean Ct*	%CV	Agreement with expected results	Mean Ct*	%CV	Agreement with expected results	Mean Ct*	%CV	Total Agreement with expected results	95% CI
Flu A Low Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%

Flu A Medium Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
Flu B Low Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
Flu B Medium Positive	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (30/30)	40.1	0.0	100% (90/90)	95.9-100%
RSV Low Positive	100% (30/30)	33.4	1.6	100% (30/30)	33.7	1.2	100% (30/30)	33.8	2.4	100% (90/90)	95.9-100%
RSV Medium Positive	100% (30/30)	31.6	1.8	100% (30/30)	32.5	2.9	100% (30/30)	31.9	3.3	100% (90/90)	95.9-100%
High Negative	100% (30/30)	40.1	0.0	90% (27/30)	39.9	1.7	96.7% (29/30)	40.1	0.4	95.6% (86/90)	89.1-98.3%
Total Agreement	100% (210/210)			98.6% (207/210)			99.5% (209/210)			99.4% (626/630)	98.4-99.8%

Note: Samples that are negative for an analyte are assigned a value of 40.1 for the purposes of statistical analysis only. Samples that are negative have a Ct value of zero (0).

*Mean Ct for RSV detection channel

Focus also assessed the reproducibility of three different lots of Positive Control Pack by testing each lot of control with one lot of reaction mix. The study yielded a total of 12 replicates per sample for each lot of reaction mix. Each lot of Positive Control was run with two replicates/run, two runs per/day for three days.

Simplexa™ Flu A/B & RSV Direct Positive Control Inter-Lot Reproducibility

	N	Min	Max	Mean	SD	%CV
Flu A	36	33.20	35.00	33.79	0.38	1.13
Flu B	36	30.10	31.10	30.35	0.22	0.74
RSV	36	30.60	35.80	33.27	0.75	2.26

b. Linearity/assay reportable range:

Not applicable

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

To assess the room-temperature stability of positive control (inactivated virus for each analyte blended together), aliquots of positive control were thawed and kept at room temperature for up to 24 hours, and were then tested on the same assay run.

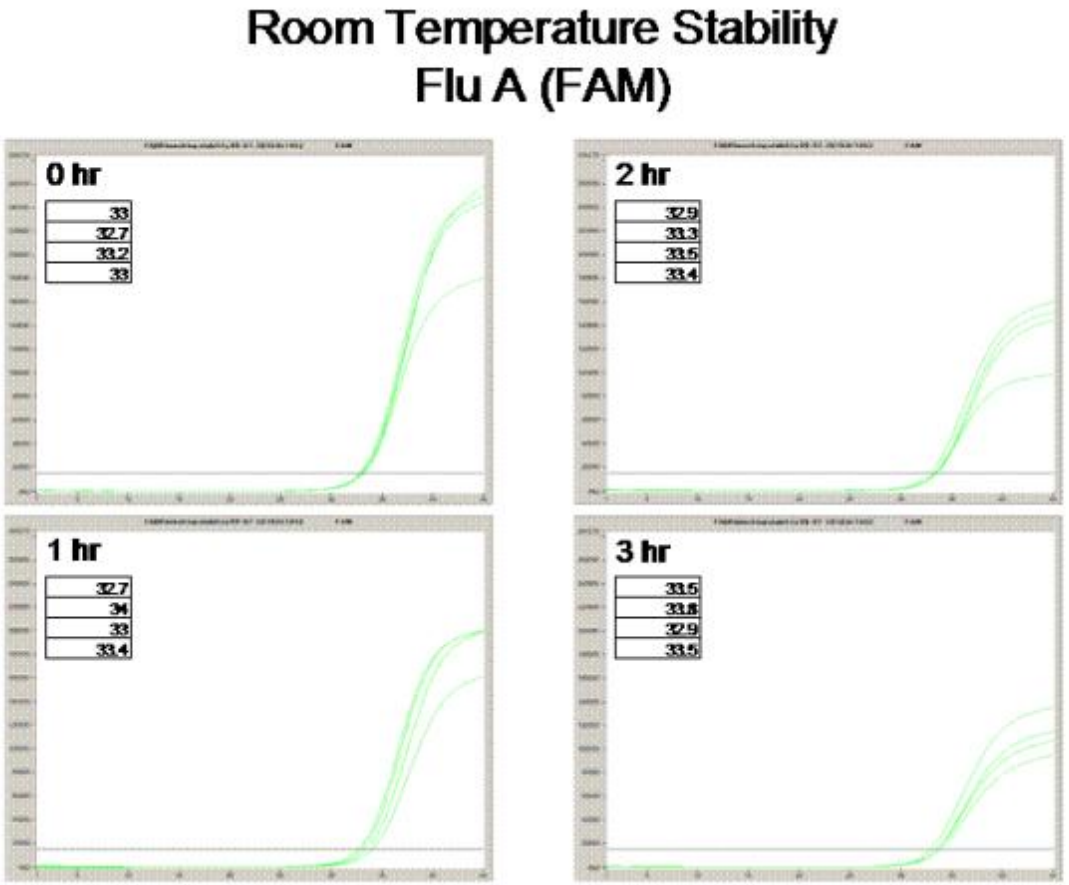
Room-temperature stability of positive control

Time at room temp	Flu A	Flu B	RSV	RNA IC
	FAM	JOE	CFR610	Q670
0 hr	35.0	32.7	32.1	33.4
	35.0	32.1	32.2	32.9
	35.2	32.6	32.0	33.1

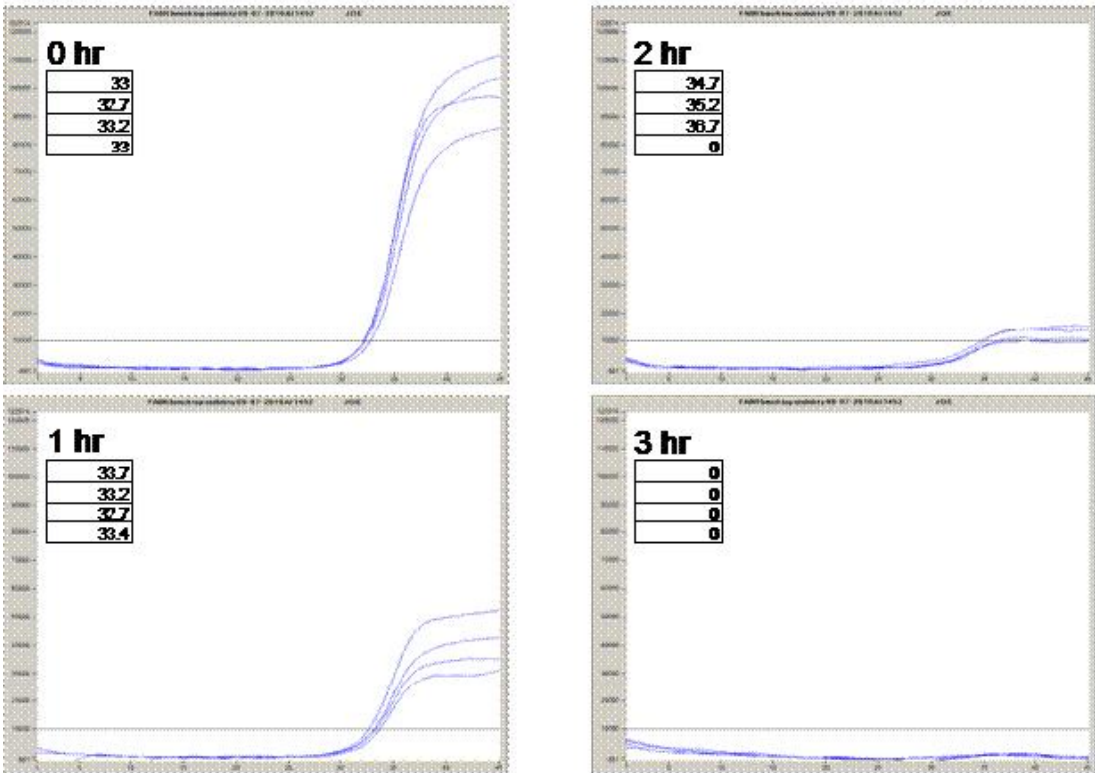
	34.7	32.8	31.7	34.0
Average Ct	35.0	32.6	32.0	33.4
24 hr	34.7	33.1	32.4	32.4
	34.8	33.1	32.6	32.6
	35.2	32.9	31.9	33.0
	34.7	33.0	32.1	32.9
Average Ct	34.9	33.0	32.3	32.7

An RNA internal control (IC) is used to monitor the extraction process and to detect RT-PCR inhibition. The RNA IC consists of encapsulated RNA template and is present in the assay reaction mix.

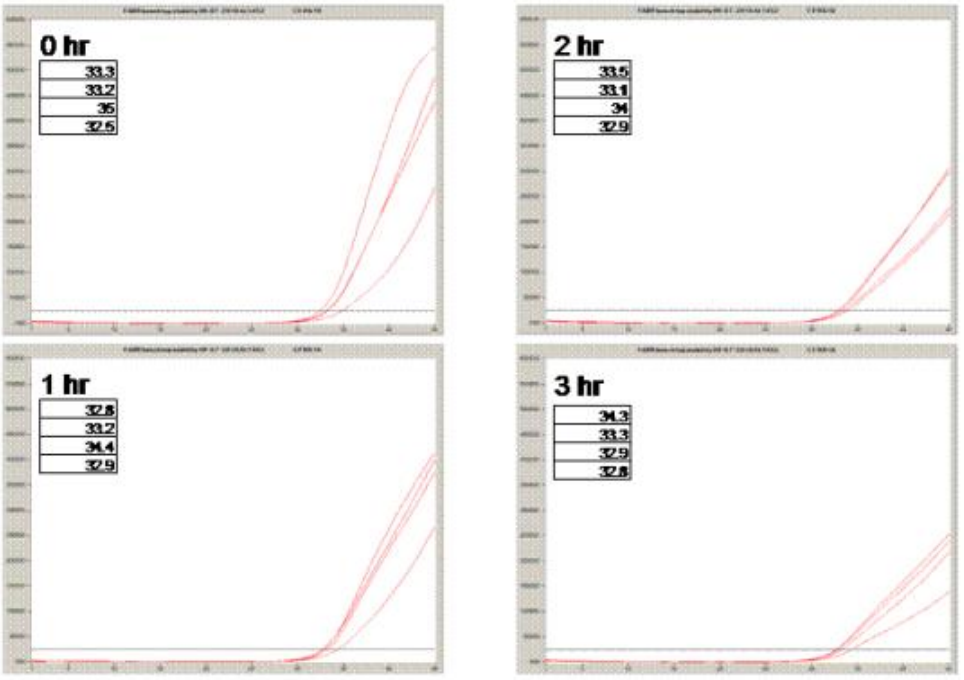
Reaction mix stability was assessed in a study where the reaction mix was prepared and then frozen. After freezing, the reaction mix was thawed and tested immediately (time 0) and every hour for three hours. The positive control was used as the sample. For each target there was a marked decrease in the amplitude of the amplification plots as time progressed. The amplitude of the curves did not diminish to the point where they did not cross the threshold until 2 hours after defrosting. Focus conservatively set the room temperature stability at 30 minutes.

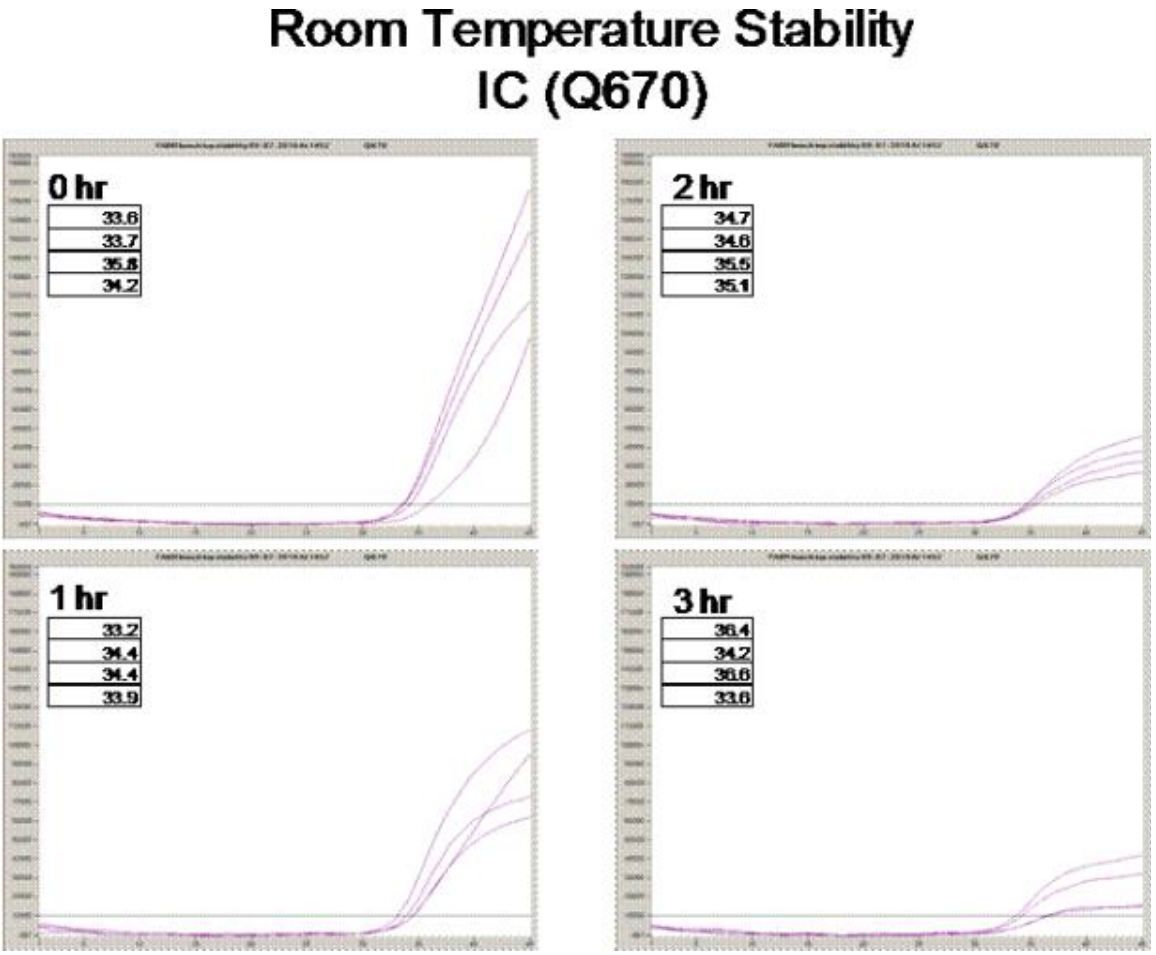


Room Temperature Stability
Flu B (JOE)



Room Temperature Stability
RSV (CFR610)





Positive control (PC) for this product is a blend of inactivated viruses for influenza A, influenza B and RSV provided in single use aliquots frozen at -20°C. The materials used in this control are the same as materials used in the control included in the Simplexa™ Flu A/B & RSV kit (k102170). Lot release criteria for the PC is a Ct score in the low to mid 30's with a lot-to-lot CV ≤10%. The PC, in conjunction with the Simplexa™ RNA IC, is used to verify reagent and system performance. The PC is meant to be a control for global failure of the assay (missing reaction component, instrument failure, etc.). A PC should be included in each run.

No Template Control (NTC): The NTC consists of Viral Transport Media and after addition of reaction mix is taken through the RNA extraction process, amplification, and detection. The NTC reaction should not exhibit fluorescence growth curves that cross the threshold line in any of the FLUA, FLUB, or RSV detection channels but must provide a valid Ct value (CT ≤40, ≠0) for the RNA IC. If any of the FLUA, FLUB, or RSV channels provide a Ct value CT ≤40, ≠0 for the NTC, contamination may have occurred in one or more components of the system. The NTC should be included in each run.

Quality control ranges have been established as indicated in the table below. If the controls are not within these parameters, patient results should be considered invalid

and the assay repeated. Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice.

Simplexa™ Flu A/B & RSV Direct Expected Control Results

Control Type	Flu A	Flu B	RSV	RNA Internal Control (RNA IC)
Simplexa™ Flu A/B & RSV Positive Control[1]	Detected	Detected	Detected	Not applicable[2]
No Template Control (NTC)	Not Detected	Not Detected	Not Detected	Valid

[1] Typical Ct values for the Positive Control range between 25 to ≤40

[2] Detection of the Simplexa™ RNA Internal Control (RNA IC) is not required for a valid result

d. Detection limit

The Limit of Detection (LoD) was determined for the Simplexa™ Flu A/B & RSV Direct assay by limiting dilution studies using viral stocks of the virus strains listed in the tables below. The specimens used for this study were contrived from verified [re-grown and re-titered] viral material provided by an outside vendor. The specimens were diluted from the viral stock material with pooled negative swab clinical matrix.

This study consisted of separate runs to evaluate the LoD of the Simplexa™ Flu A/B & RSV Direct kit; multiple runs were performed to determine the tentative LoD of three strains of seasonal influenza A, two strains of seasonal influenza B and two strains of RSV. Tentative LoD was confirmed by assaying 20 replicates of each dilution identified as the tentative LoD.

To determine tentative LoD, each of the seven strains were individually spiked into negative swab matrix and serially diluted. Four different dilutions around the theoretical LoD were tested. Each individual level was assayed in quadruplicate. The lowest concentration at which all four replicates are positive is treated as the tentative LoD.

Confirmation of LoD was determined over multiple runs. Each strain was spiked into negative swab matrix at the concentration of tentative LoD. Each sample was tested 20 times. A single extraction of Positive Control (PC) and No Template Control (NTC) was included in each day of testing. At least 19 of the 20 replicates (95%) must be detected to confirm the LoD.

Simplexa™ Flu A/B & RSV Direct Limit of Detection

Viral Strain	Concentration $TCID_{50}$/mL	# of Detected/Total	
		Screening	Confirmation
Influenza A/Hong Kong/8/68 H3N2	5	4/4	17/20
	10	4/4	20/20
	20	4/4	-
	40	4/4	-
Influenza A/PR/8/34 H1N1	0.005	4/4	20/20
	0.01	4/4	-
	0.02	4/4	-
	0.04	4/4	-
Influenza A/Swine NY/02/2009 H1N1	0.025	0/3	-
	0.05	3/4	-
	0.1	4/4	20/20
	0.2	4/4	-
	0.4	4/4	-
Influenza B/Great Lakes/1739/54	1	3/4	-
	2	4/4	20/20
	4	4/4	-
	8	4/4	-
Influenza B/Malaysia/2506/2004	5	2/4	-
	10	3/4	-
	20	4/4	19/20
	40	4/4	-
RSV A2	1	4/4	19/20*
	2	4/4	20/20
	3	4/4	-
	4	4/4	-
RSV B CH93-18(18)	2	3/4	-
	3	4/4	20/20
	4	4/4	-
	6	4/4	-

* "Insufficient Volume" flag was generated for one replicate in the first run of eight replicates therefore an extra replicate was set up on a later run.

Simplexa™ Flu A/B & RSV Direct Limit of Detection Summary

Simplexa™ FluA/B & RSV Direct Assay – Limit of Detection	
Viral Strain	Concentration ($TCID_{50}$/mL)
Influenza A/Hong Kong/8/68 H3N2	10
Influenza A/PR/8/34 H1N1	0.005
Influenza A/Swine NY/02/2009 H1N1	0.1
Influenza B/Great Lakes/1739/54	2
Influenza B/Malaysia/2506/2004	20
RSV A2	1
RSV B CH93-18(18)	3

e. Analytical reactivity

Cross-Reactivity

The panel consisted of 32 potential cross-reactants individually spiked at clinically

relevant concentrations into pooled negative swab clinical matrix. Concentration of the cross reactants were determined by growing and titering the organisms listed. The unspiked matrix was also tested to serve as a baseline. Specimens were tested in triplicate to screen for cross-reactivity. If influenza A, influenza B or RSV was detected in any of the three replicates, an additional five replicates were tested for confirmation.

Simplexa™ Flu A/B & RSV Direct Cross-Reactivity Results

Cross-Reactant	Concentration	#Detected/#Total		
		Flu A	Flu B	RSV
Adenovirus 1	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Adenovirus 7A	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Bordetella pertussis A639	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Chlamydia pneumoniae[1]	1.00 x 10^6 copies/mL	0/3	0/3	0/3
Cytomegalovirus (CMV)	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Coronavirus 229E	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Coronavirus OC43	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Corynebacterium diphtheriae	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
E. coli O157	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
EBV[2]	1.00 x 10^5 copies/mL	0/3	0/3	0/3
Enterovirus 71	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Haemophilus influenzae	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Lactobacillus plantarum, 17-5	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Legionella longbeachae	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Measles	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Metapneumovirus	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Moraxella catarrhalis Ne11	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Mumps	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Mycobacterium tuberculosis	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Mycoplasma pneumoniae M129	1.00 x 10^6 $TCID_{50}$/mL	0/3	0/3	0/3
Neisseria elongata	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Neisseria meningitidis	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Parainfluenza type 1	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Parainfluenza type 2	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Parainfluenza type 3	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Pseudomonas aeruginosa	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Rhinovirus 1A	1.00 x 10^5 $TCID_{50}$/mL	0/3	0/3	0/3
Staphylococcus aureus, COL	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Staphylococcus epidermidis	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Streptococcus pneumoniae	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Streptococcus pyogenes	1.00 x 10^6 cfu/mL	0/3	0/3	0/3
Streptococcus salivarius	1.00 x 10^6 cfu/mL	0/3	0/3	0/3

1) *C. pnuemoniae* material used had a titer of 1.52 x 10^5 IFU/mL, it was further quantified using a real-time qPCR assay.
2) EBV was quantified by the vendor using a real time qPCR assay.

Reactivity

The following strains were tested for analytical reactivity in addition to the seven strains tested in the LoD studies. Each strain was assayed in triplicate to provide an estimate of LoD. If 3/3 replicates did not provide a positive result in the correct channel, an additional dilution to provide a two-fold higher level of virus was created and tested. The following table summarizes the additional strains of virus tested for reactivity and the level of each strain of virus that provided 3/3 positive results. Ct

values obtained during testing indicate all viral strains were tested near the LoD.

Simplexa™ Flu A/B & RSV Direct - Reactivity with Additional Strains

Strain	Concentration Level (TCID50/mL)	Result
Influenza A Brisbane/10/07 H3	1.00×10^2	Flu A Detected
Influenza A Brisbane/59/07 H1	1.00×10^2	Flu A Detected
Influenza A New Caledonia/20/99 H1N1	1.00×10^2	Flu A Detected
Influenza A Port Chalmers/1/73 H3N2	1.00×10^2	Flu A Detected
Influenza A Solomon Island/03/06 H1	1.00×10^2	Flu A Detected
Influenza A Swine NY/02/2009 H1N1	1.00×10^2	Flu A Detected
Influenza A Taiwan/42/06 H1N1	1.00×10^2	Flu A Detected
Influenza A Wisconsin/67/05 H3	1.00×10^2	Flu A Detected
Influenza A/WS/33 H1N1	1.00×10^2	Flu A Detected
Influenza A/CA/7/2009 NYMC x-179A	1.00×10^2	Flu A Detected
Influenza A Swine H1N1/Iowa/15/1930 (Tissue culture adapted)	1.00×10^3	Flu A Detected
Influenza A Swine H1N1/USA/1976/1931 (Tissue culture adapted)	1.00×10^3	Flu A Detected
Influenza A/PR8 Vietnam/1203/2004 (H5N1 – inactivated virus)	Unknown	Flu A Detected
Influenza B Allen/45	1.00×10^2	Flu B Detected
Influenza B Florida/02/2006 (Victoria)	1.00×10^2	Flu B Detected
Influenza B Florida/04/2006 (Yamagata)	1.00×10^2	Flu B Detected
Influenza B Florida/07/04 (Yamagata)	1.00×10^2	Flu B Detected
Influenza B Hong Kong/5/72 (Victoria)	1.00×10^2	Flu B Detected
Influenza B Lee/40	1.00×10^2	Flu B Detected
Influenza B Maryland/1/59	1.00×10^2	Flu B Detected
Influenza B Panama/45/90 (Yamagata)	1.00×10^2	Flu B Detected
Influenza B Taiwan/2/62	1.00×10^3	Flu B Detected
RSV A Long	1.00×10^2	RSV Detected
RSV B 9320	1.00×10^2	RSV Detected
RSV B Wash/18537/62	1.00×10^2	RSV Detected
RSV B WV/14617/85	1.00×10^2	RSV Detected

f. Interference studies:

Interfering Substances

Potentially interfering or inhibitory substances that may be present in nasopharyngeal swabs or interfere with the PCR reaction were evaluated for the viral strains indicated below. A low level of virus was spiked into pooled negative clinical matrix that was screened to be negative for influenza A, influenza B and RSV. This low pool was evaluated for baseline performance. Potentially interfering substances were spiked into the low sample pool at the indicated concentrations in the following tables. All strains were tested at two to four times the LoD.

Low Pool Concentrations

Virus	Strain	Concentration
Influenza A	Influenza A/PR/8/34 H1N1	0.01 $TCID_{50}$/mL
Influenza B	Influenza B/Malaysia/2506/2004	40 $TCID_{50}$/mL

RSV	RSV A2	4 $TCID_{50}$/mL

Interfering Substances Results

Potential Interferent	Active Ingredient	Interferent Concentration	#Detected/#Total		
			Flu A	Flu B	RSV
Afrin nasal spray	Oxymetazoline	15% (v/v)	3/3	3/3	3/3
Antibacterial, systemic	Tobramycin	4 µg/mL	3/3	3/3	3/3
Antibiotic, nasal ointment	Mupirocin	6.6 mg/mL	3/3	3/3	3/3
Blood	N/A	2% (v/v)	3/3	3/3	3/3
Purified mucin protein	Bovine submaxillary gland type I-S	60 µg/mL	3/3	3/3	3/3
Nasal corticosteroid – Beconase AQ	Beclomethasone	5% (v/v)	3/3	3/3	3/3
Nasal Corticosteroid – Fluticasone	Fluticasone	5% (v/v)	3/3	3/3	3/3
Relenza antiviral drug	Zanamavir	3.3 mg/mL	3/3	3/3	3/3
Tamiflu antiviral drug	Oseltamivir	1 µM	3/3	3/3	3/3
Zicam nasal gel	Luffa Opperculata, Galphimia glauca, histanium hydrochloricum	5% (v/v)	3/3	3/3	3/3

Interfering Microorganisms

The Simplexa Flu A/B & RSV assay was evaluated by testing the ability to identify influenza A virus, influenza B virus, and RSV when potentially inhibitory organisms are present. The panel of 32 potentially inhibitory organisms was individually spiked into a pool with a low concentration (approximately two times LoD) of influenza A (Influenza A/PR/8/34 H1N1), influenza B (Influenza B/Malaysia/2506/2004) and RSV (A2). Specimens were tested in triplicate to screen for inhibition. If signal was not detected in any detection channel (Flu A, Flu B, RSV) in any of the three replicates, an additional five replicates were tested for confirmation.

For two of the organisms tested, coronavirus and enterovirus, one of the initial three replicates was not detected, indicating a possibility of inhibition. Those specimens were tested in an additional five replicates. No inhibitory effects were confirmed for influenza A, influenza B, or RSV at the concentrations tested.

Interfering Microorganisms Results

Microorganism	Microorganism Concentration	#Detected/#Total		
		Flu A	Flu B	RSV
Adenovirus 1	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Adenovirus 7A	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Bordetella pertussis A639	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Chlamydia pneumoniae[1]	1.00 x 10^6 copies/mL	3/3	3/3	3/3

Microorganism	Microorganism Concentration	#Detected/#Total		
		Flu A	Flu B	RSV
Cytomegalovirus (CMV)	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Coronavirus 229E	1.00 x 10^5 $TCID_{50}$/mL	7/8	7/8	8/8
Coronavirus OC43[2]	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Corynebacterium diphtheriae	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
E. coli O157	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
EBV[3]	1.00 x 10^5 copies/mL	3/3	3/3	3/3
Enterovirus 71[2]	1.00 x 10^5 $TCID_{50}$/mL	7/8	7/8	8/8
Haemophilus influenzae	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Lactobacillus plantarum, 17-5	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Legionella longbeachae	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Measles	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Metapneumovirus	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Moraxella catarrhalis Ne11	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Mumps	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Mycobacterium tuberculosis	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Mycoplasma pneumoniae M129	1.00 x 10^6 $TCID_{50}$/mL	3/3	3/3	3/3
Neisseria elongata	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Neisseria meningitidis	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Parainfluenza type 1	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Parainfluenza type 2	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Parainfluenza type 3	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Pseudomonas aeruginosa	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Rhinovirus 1A	1.00 x 10^5 $TCID_{50}$/mL	3/3	3/3	3/3
Staphylococcus aereus, COL	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Staphylococcus epidermidis	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Streptococcus pneumoniae	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Streptococcus pyogenes	1.00 x 10^6 cfu/mL	3/3	3/3	3/3
Streptococcus salivarius	1.00 x 10^6 cfu/mL	3/3	3/3	3/3

1) The *C. pneumoniae* used had a titer of 1.52 x 10^5 IFU/mL it was further quantified using a real-time qPCR assay.
2) Initial testing appeared to show possible inhibition, upon repeat testing there was no evidence of inhibition.
3) EBV was quantified by the vendor using a real-time qPCR assay.

Competitive Interference

The Competitive Interference study evaluated the effects of clinically relevant co-infections with each of the analytes probed by the assay. The study assessed whether a high concentration of one virus in the specimen could potentially affect the Simplexa assay performance for another target present at low levels in the multiplex assay. A low sample ("Baseline Strain") was contrived at approximately two to four times the LoD for each target (influenza A, influenza B and RSV), and a baseline Ct was determined for each sample. Each potential concomitant infecting virus was spiked into the low level specimen according to the table below. Interference studies indicate there is no interference when two viruses are present at the concentrations tested; however clinical line data suggests very high levels of influenza A may interfere with the detection of very low concentrations of influenza B or RSV.

Competitive Interference Results

Baseline Strain	Baseline	Competitive	Competitive	Result (#Detected/#Tested)

	Concentration ($TCID_{50}$/mL)	Interferent Strain	Interferent Concentration ($TCID_{50}$/mL)	Flu A	Flu B	RSV
Influenza A/PR/8/34 H1N1	0.01	Influenza B/GL/1739/54	19566.3	3/3	3/3	0/3
	0.01	RSV A2	806	8/8	0/8	8/8
	0.01	RSV B Ch93-18(18)	10659	6/8	0/8	8/8
Influenza B/GL/1739/54	8	Influenza A/PR/8/34 H1N1	0.174	3/3	3/3	0/3
	8	RSV A2	80.6	0/3	3/3	3/3
	8	RSV B Ch93-18(18)	1065.9	0/3	3/3	3/3
RSV A2	4	Influenza A/PR/8/34 H1N1	0.174	3/3	0/3	3/3
	4	Influenza B/GL/1739/54	19566.3	0/3	3/3	3/3
RSV B Ch93-18(18)	6	Influenza A/PR/8/34 H1N1	0.174	3/3	0/3	3/3
	6	Influenza B/GL/1739/54	19566.3	0/8	8/8	7/8

g. Assay cut-off:

Assay cut-off was determined by analysis of the Limit of Detection study and clinical data generated during assay verification testing. Forty-five (45) cycles of amplification were performed during assay development to allow for the appropriate determination of assay cut off. Subsequently, verification studies were conducted with 40 cycles of amplification. The Limit of Detection for each target strain is defined as the lowest concentration of sample where ≥ 95% of 20 replicates are detected. Data analysis of the Limit of Detection study shows that the range of Ct values for influenza A, influenza B and RSV specimens at the Limit of Detection (LoD) were <40. Review of clinical data show that the >95% of the positive specimens have a Ct value ≤35: 7 out of 207 positive specimens gave Ct value of >35. Specimens that were positive for influenza A had Ct values in the range of 18.3 to 35.2. Specimens that were positive for influenza B had Ct values in the range of 20.5 to 37.1. Specimens that were positive for RSV had Ct values in the range of 21.8 to 37.1. Based on the available data, the assay cut off was set at Ct = 40 for each detector.

h. Carryover Contamination:

The carry-over study searched for the presence of contamination in negative specimens. The study was designed by alternately placing high positive and negative specimens on each disc. A total of 60 negative specimens and 60 high positive specimens were tested across 17 runs using four different instruments. The sample size of 60 was chosen to provide lower bound of the 95 CI > 90% for the negative proportion of the negative sample in absence of carry-over contamination effect.

The carryover effect was evaluated by comparing the observed negative rate for the negative sample. The 95% confidence interval for % negative of negative sample was calculated. All sixty (60) replicates of negative sample were "Not Detected" for all three target viruses. Therefore no evidence of carryover was observed.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

c. Fresh versus Frozen comparison:

A panel of 180 contrived samples was created using multiple strains of influenza A, influenza B and RSV. The panel was tested fresh. After testing the panel was frozen for a period of five days; the samples were then defrosted for an hour and then refrozen. The refrozen panel was defrosted and tested again in the Simplexa™ Flu A/B & RSV Direct assay. The results from all 180 frozen panel members were concordant with the results when tested fresh.

Fresh vs. Frozen Results

Strain	Fresh Interpretation	Frozen Interpretation			
		Flu A Detected	Flu B Detected	RSV Detected	Total
Influenza A/HK/8/68 (H3N2)	Flu A Detected	30			30
Influenza A/PR/8/34 (H1N1)	Flu A Detected	30			30
Influenza B/Flordia/04/2006	Flu B Detected		18		18
Influenza B/GL/1739/54	Flu B Detected		12		12
Influenza B/Malaysia/2506/2004	Flu B Detected		12		12
Influenza B/Panama/45/90	Flu B Detected		18		18
RSV A2	RSV Detected			30	30
RSV B CH93-18(18)	RSV Detected			30	30
Total		60	60	60	180

In addition, Passing-Bablok regression analysis was performed comparing the fresh to the frozen results. The slope of the regression line is very near 1.0 for each target, the confidence interval around the slope encompasses 1.0.

Passing-Bablok Regression Analysis

Target	Parameter	Estimate	95% Confidence Interval
Flu A	Slope	0.99	(0.9565,1.0102)

	Intercept	0.53	(-0.1418,1.3913)
Flu B	Slope	1.03	(1.0000,1.0779)
	Intercept	-0.82	(-2.2838,0.0000)
RSV	Slope	1.00	(0.9762,1.0185)
	Intercept	-0.20	(-0.6880,0.5036)

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Three external testing sites and one internal site participated in the prospective clinical study. Reference results for influenza A, influenza B viruses and respiratory syncytial virus were generated using culture. Culture results were carried forward from the results obtained at the time of sample collection. A total of 722 nasopharyngeal swabs specimens were obtained from prospectively collected specimens from patients with signs and symptoms of viral respiratory tract infection and distributed randomly to testing sites. Prospective samples were collected in Eastern United States from 10-November-2010 to 11-March-2011, the Mid-Western United States from 08-January-2011 to 02-February 2011 and in Australia from 17-August-2010 to 20-October-2010. Of the 722 specimens, 325 specimens were collected from female patients and 397 specimens were collected from male patents. A total of 327 specimens were from patients <5 years of age, 223 specimens were from patients between 5-22 years of age, 158 specimens were from patients between 22 to 60 years of age and 14 specimens were from patients >60 years of age. One sample was excluded from the prospective analysis due to Invalid result for Flu A, three samples were excluded due to an invalid result for Flu B and one sample was excluded due to an invalid result for RSV. The percentage of specimens with invalid results during the study was 0.4% (3/722) with a 95% CI of 0.1% to 1.2%

Clinical Agreement – Flu A (Prospective – All Sites Combined)

Culture Result		Simplexa™ Results – Flu A		Sensitivity/Specificity 95% CI
	N	Detected	Not Detected	
Detected	68	66	2	**Sensitivity:** 97.1%(66/68) 95% CI: 89.9 to 99.2%
Not Detected	653	14	639	**Specificity:** 97.9%(639/653) 95% CI: 96.4 to 98.7%

Clinical Agreement – Flu B (Prospective – All Sites Combined)

Culture Result		Simplexa™ Results – Flu B		Sensitivity/Specificity 95% CI
	N	Detected	Not Detected	
Detected	21	21	0	**Sensitivity:** 100.0%(21/21) 95% CI: 84.5 to 100.0%
Not Detected	698	1	697	**Specificity:** 99.9%(697/698) 95% CI: 99.2 to 100.0%

Clinical Agreement – RSV (Prospective – Site 1)

Culture Result		Simplexa™ Results – RSV		Sensitivity/Specificity 95% CI
	N	Detected	Not Detected	
Detected	1	1	0	**Sensitivity:** 100.0%(1/1) 95% CI: 20.7 to 100.0%
Not Detected	329	6[a]	323	**Specificity:** 98.2%(323/329) 95% CI: 96.1 to 99.2%

[a] 4/6 samples were confirmed as RSV positive by an FDA cleared NAT

Clinical Agreement – RSV (Prospective – Site 2)

Culture Result		Simplexa™ Results – RSV		Sensitivity/Specificity 95% CI
	N	Detected	Not Detected	
Detected	73	72	1[a]	**Sensitivity:** 98.6%(72/73) 95% CI: 92.6 to 99.8%
Not Detected	172	18[b]	154	**Specificity:** 89.5%(154/172) 95% CI: 84.1 to 93.3%

[a] 1/1 sample confirmed as RSV positive by an FDA cleared DFA

[b] 11/18 samples were confirmed as RSV positive by an FDA cleared DFA

Clinical Agreement – RSV (Prospective – Site 3)

Culture Result		Simplexa™ Results – RSV		Sensitivity/Specificity 95% CI
	N	Detected	Not Detected	
Detected	10	9	1	**Sensitivity:** 90.0%(9/10) 95% CI: 59.6 to 98.2%
Not Detected	136	21[a]	115	**Specificity:** 84.6%(115/136) 95% CI: 77.5 to 89.7%

[a] 20/21 samples were confirmed as RSV positive by an FDA cleared NAT

Three external testing sites and one internal testing site participated in a retrospective Clinical Agreement study. Reference results for influenza A, influenza B viruses and respiratory syncytial virus were generated using culture. Culture results were carried forward from the results obtained at the time of sample banking. A total of 223 nasopharyngeal swabs specimens were obtained from retrospectively banked specimens from patients with signs and symptoms of viral respiratory tract infection.

Clinical Agreement – Flu A (Retrospective – All Sites Combined)

Culture Result		Simplexa™ Results – Flu A		PPA/NPA 95% CI
	N	Detected	Not Detected	
Detected	79	76	3	**PPA:** 96.2% (76/79) 95% CI: 89.4 to 98.7%
Not Detected	144	1	143	**NPA:** 99.3% (143/144) 95% CI: 96.2 to 99.9%

* PPA = Positive Percent Agreement, NPA = Negative Percent Agreement

Clinical Agreement – Flu B (Retrospective – All Sites Combined)

Culture Result		Simplexa™ Results – Flu B		PPA/NPA 95% CI
	N	Detected	Not Detected	
Detected	41	40	1	**PPA:** 97.6% (40/41) 95% CI: 87.4 to 99.6%

Not Detected	182	0	182	**NPA:** 100% (182/182) 95% CI: 97.9 to 100.0%

* PPA = Positive Percent Agreement, NPA = Negative Percent Agreement

Clinical Agreement – RSV (Retrospective – All Sites Combined)

Culture Result		Simplexa™ Results – RSV		PPA/NPA 95% CI
	N	Detected	Not Detected	
Detected	12	12	0	**PPA:** 100.0% (12/12) 95% CI: 75.7 to 100.0%
Not Detected	211	3	108	**NPA:** 98.8% (208/211) 95% CI: 95.9 to 99.5%

* PPA = Positive Percent Agreement, NPA = Negative Percent Agreement

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:
   Prospective specimens used in the clinical study were obtained from the United States and Australia. The prevalence of all influenza viruses in the US during the September 2010 to March 2011 collection period ranged from 2.1 to 35.5%[1]. During the season, among influenza positives, 72.9% were positive for Influenza A and 27.1 % for Influenza B. The positivity rate for RSV in the United States during the period that included the September 2010 to March 2011 collection period was 15.9%[2]. In Australia during the 2010 influenza season, 9% of specimens have tested positive for influenza; the positivity rate for RSV was not reported[3].

   [1] http://www.cdc.gov/flu/weekly/pastreports.htm
   [2] http://www.cdc.gov/surveillance/nrevss/rsv/state.html
   [3] http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-ozflu-no44-10.htm

Prevalence (Positives as Determined by Reference Method) Observed During the Prospective Clinical Study Stratified by Collection Sites

Australia (n=330)					
Analyte	**Total (Prevalence)**	**< 5 years (n=63)**	**5-21 years (n=106)**	**22-60 years (n=147)**	**> 60 years (n=14)**
Flu A	2.7% (9/330)	0	5	4	0
Flu B	2.9% (7/330)	3	2	2	0
RSV	0.7% (11/330)	0	0	1	0

Ohio (n=245)					
Analyte	**Total (Prevalence)**	**< 5 years (n=195)**	**5-21 years (n=48)**	**22-60 years (n=2)**	**> 60 years (n=0)**
Flu A	11.4% (28/245)	14	14	0	0
Flu B	1.2% (3/245)	1	2	0	0

RSV	29.8% (73/245)	70	3	0	0

Virginia (n=147)					
Analyte	**Total (Prevalence)**	**< 5 years (n=69)**	**5-21 years (n=69)**	**22-60 years (n=9)**	**> 60 years (n=0)**
Flu A	21% (31/147)	10	20	1	0
Flu B	7.5% (11/147)	1	10	0	0
RSV	7.5% (11/147)	9	2	0	0

N. Instrument Name:

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

O. System Descriptions:

1. Modes of Operation:

   The system includes the computer, related peripherals, handheld barcode scanner, Integrated Cycler, Integrated Cycler Studio software and operator manual. The Integrated Cycler is a Real-Time Polymerase Chain Reaction (PCR) thermocycler used for detection of nucleic acid from prepared biological samples. The instrument utilizes disc media to contain and to process samples and real-time fluorometric detection to identify targets within the sample wells. The instrument's operation parameters are controlled by the use of an external personal computer and associated software. The universal amplification disc can process up to 96 independent samples while the direct amplification disc can process up to eight samples.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X____ or No _____

3. Specimen Identification:

   Specimens ID's are manually entered into the user interface by the user and the sample ID is matched with the corresponding location on the consumable.

4. Specimen Sampling and Handling:

   Liquid samples from nasopharyngeal swabs in UTM are manually transferred into the consumable and sealed using the attached adhesive backed cover tape.

5. Calibration:

Calibration is not recommended.

6. Quality Control:

   An MS2 phage is added to each sample as an internal standard and indicates the quality of the assay results. The algorithm for incorporating the internal standard data into the reported assay result is shown above in Part 1 Mode of operation in the Software section.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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