510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K102170

B. Purpose for Submission:

This is a new 510k 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 using nucleic acid isolation and amplification. The isolation and purification of the viral RNA is performed using either the MagNA Pure LC Instrument (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or the NucliSENS® easyMAG™ System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux). The amplification and detection is performed on the 3M Integrated Cycler with Integrated Cycler Studio Software version 2.1 or higher.

E. Applicant:

Focus Diagnostics, Inc.

F. Proprietary and Established Names:

Simplexa™ Flu A/B & RSV

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980

2. Classification:

   Class II

3. Product code:

   OCC

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Focus Diagnostics Simplexa™ Flu A/B & RSV 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 (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 during the 2010 influenza season 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 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.

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 2.1 or higher and either a Roche MagNA Pure LC Instrument (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS® easyMAG™ System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux).

I. Device Description:

The Focus Diagnostics Simplexa™ Flu A/B & RSV assay is a multiplex Real-Time RT-PCR test that detects and differentiates influenza A ,influenza B, and RSV RNA from nasopharyngeal swabs from human patients with signs and symptoms of viral respiratory tract infection.

Patient specimens are collected and placed in sterile viral transport medium containing protein stabilizer, antibiotics to inhibit bacterial and fungal growth, and buffer solution. An Internal Control (RNA IC) is added to each sample prior to nucleic acid extraction by either the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or the Automated Magnetic Extraction Reagents (bioMérieux). In the case of the MagNA Pure kit, it is recommended that extraction be carried out with qualified MagNA Pure reagent lots only; a list of these qualified extraction reagents will be maintained at www.focusdx.com.

A sample of the extracted nucleic acid is added to the Simplexa™ Flu A/B & RSV assay reagents that contain four sets of a primer and a bi-functional fluorescent probe-primer. Each set of primer and bi-functional fluorescent probe-primer recognizes a specific target, namely, influenza A matrix gene, influenza B matrix gene, RSV M gene, and RNA IC. PCR amplification is carried out on the 3M Integratede Cycler. The fluorescence output is is analyzed and test results are determined using the Integrated Cycler Studio Software.
The Simplexa™ Flu A/B & RSV kit contains Primer Mix (PM), Master Mix (MM), RT Mix (RT), RNA Internal Control (RNA IC), No Template Control (NTC), Flu A/B & RSV Positive Control (PC), Simplexa™ Flu A/B & RSV Barcode Card, and Package Insert.

Simplexa™ Flu A/B & RSV Gene targets and Probe Labels:

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak (nm)	Emission Peak (nm)
Influenza A	Matrix	FAM	495	520
Influenza B	Matrix	JOE	520	548
RSV	M gene	CFR610	590	610
Internal Control (RNA IC)	N/A	Q670	644	670

Interpretation of Sample Results:

Reporting results is a three step process.

1. Determine if the run is valid by examining the Flu A/B & RSV Positive Control, No Template Control, and RNA Internal Control.

Criteria for a Valid Control (simplified)*

Control	Flu A Ct	Flu B Ct	RSV Ct	RNA IC Ct
No Template Control	0	0	0	≤40, ≠0
Positive Control	≤40, ≠0	≤40, ≠0	≤40, ≠0	Not Applicable (N/A)

* See notes below for full description.

a. No Template Control:

i. If the No Template Control is Positive (Ct value ≤40, ≠0 for Flu A, Flu B or RSV), then this indicates possible contamination of prepared samples. The control is invalid and all patient specimens must be re-assayed.

ii. If the RNA IC is not detected in the No Template Control, the assay run is invalid and all patient specimens must be re-assayed.

iii. If the No Template Control is Negative for Flu A, Flu B or RSV detector (Ct = 0) AND If the RNA IC is detected for the No Template Control, then this control is valid and acceptable.

b. Positive Control

i. If the Positive Control result is a Ct = 0 for Flu A, Flu B or RSV, the assay run is considered invalid and unacceptable. All patient specimens must be re-assayed.

ii. If the Ct values for Flu A, Flu B and RSV A are ≤40, ≠0 the assay run is considered valid and acceptable.

c. RNA Internal Control:

i. Detection of the Simplexa™ RNA Internal Control is required to report a negative result.

ii. Detection of the Simplexa™ RNA Internal Control is not required to report a positive result.

2. Examination of Patient Specimen Results.

Examination of clinical specimen results should be performed after the Positive and No Template Controls have been examined and determined to be valid and acceptable. Flu A, Flu B, RSV and RNA IC results must be examined for each patient specimen.

a. Amplification plots should be examined for every result with a "Data Quality" message. From the Data tab, the user selects the curve to review and then clicks Refresh. The software will draw the selected curves and adjust the scale of the graph. A valid amplification curve shows a smooth, exponential increase. An invalid amplification curve may be a non-exponential or linear curve or a curve with data "spikes" where the curve may cross the threshold. If the curve is valid after examination, the Ct value reported may be used to determine if Flu A, Flu B, or RSV targets are detected.

b. If the amplification curve is valid for Flu A, Flu B, or RSV, the RNA IC is not required to be detected to report a positive result.

3. Interpretation of Results.

The interpretation of the assay specimen results is as follows:

Example	Flu A Ct value	Flu B Ct value	RSV Ct value	RNA IC Ct value*	Interpretation
1	0	0	0	≤40, ≠0	Flu A, Flu B and RSV Not Detected
2	≤40, ≠0	0	0	N/A	Flu A Detected
3	0	≤40, ≠0	0	N/A	Flu B Detected
4	0	0	≤40, ≠0	N/A	RSV Detected
5	≤40, ≠0	≤40, ≠0	0	N/A	Flu A, Flu B Detected
6	0	≤40, ≠0	≤40, ≠0	N/A	Flu B, RSV Detected
7	≤40, ≠0	0	≤40, ≠0	N/A	Flu A, RSV Detected
8	≤40, ≠0	≤40, ≠0	≤40, ≠0	N/A	Flu A, Flu B and RSV Detected
9	0	0	0	0	Invalid, re-extract and repeat.

Ct = cycle threshold. Detected is a Ct ≤40, ≠0. Not Detected is a Ct = 0, * Detection of the Simplexa™ RNA Internal Control is not required to be detected to report a positive result.

J. Substantial Equivalence Information:

1. Predicate device name(s): Prodesse ProFlu+ Assay

2. Predicate Numbers (s): K092500 , K081030, K073029

3. Comparison with predicate:

Similarities

Device Characteristics	Simplexa™ Flu A/B & RSV	Prodesse ProFlu+
Intended Use	The Focus Diagnostics Simplexa™ Flu A/B & RSV 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 (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 during the 2010 influenza season 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 Influenza A virus is suspected based on current	The ProFlu™+ Assay is a multiplex Real-Time PCR (RT-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 (NP) 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 criteria recommended by public health authorities, specimens should be collected with appropriate

Device Characteristics	Simplexa™ Flu A/B & RSV	Prodesse ProFlu+
	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.	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.
510(k)	K102170	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 fromInfluenza A, Influenza B, and RSV
Sample Types	Nasopharyngeal swabs	Nasopharyngeal swabs
Extraction Methods	Roche MagNA Pure LC System, bioMérieux NucliSENS easyMAG	Roche MagNA Pure LC System, bioMérieux NucliSENS easyMAG
Assay Type	Real-Time RT-PCR based system	Real-Time RT-PCR based
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 Viral Target	Matrix gene	Matrix gene

Differences

Device Characteristics	Simplexa™ Flu A/B & RSV	Prodesse ProFlu+
Influenza B Viral Target	Matrix gene	Non-structural NS1 and NS2

Device Characteristics	Simplexa™ Flu A/B & RSV	Prodesse ProFlu+
RSV Target	M gene	Polymerase
Assay Instrument	3M Integrated Cycler	Cepheid SmartCycler II System

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

- Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s, August 12, 2005
- Guidance for Industry and FDA Staff: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path, May 1, 2007
- Draft Guidance for Industry and FDA Staff: Establishing Performance Characteristics of In Vitro Diagnostic Devices for Detection or Detection and Differentiation of Influenza Viruses, February 12, 2008
- Guidance for Industry and FDA Staff: Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay, October 9, 2009

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 RNA 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 the separation of the fluorophore from the quencher as a result of the binding of a probe element to the extended RNA fragment synthesized during amplification.

The 3M Integrated Cycler is a rapid real-time Polymerase Chain Reaction thermocycler used for the identification of nucleic acid from prepared biological samples. The instrument utilizes disk media to contain and to process samples. The instrument uses real time flourometric detection to identify targets within the sample wells. The instrument is controlled by an external computer running the Integrated Cycler Studio Software. Together, the instrument, software and test kit are referred to as the "Microfluidic Molecular System."

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Inter-lot Precision

   Inter-lot precision was assessed by testing three (3) samples and a positive control for each target across three different lots. The panel of samples consisted of high negative, low positive, and moderately positive samples for each of influenza A, influenza B, and RSV. The samples were generated from swabs and were run in triplicate. One operator at one study site made one extraction of each sample and tested across lots.

   The variability attributable to lot-to-lot imprecision was found by fitting the appropriate general linear mixed model, with "lot" as a random variable, and partitioning out the variance. Total variability was split into inter-lot variability, and intra-lot variability (the unexplained variability, or error term of the model). The inter-lot variability component is summarized in the tables below. Imprecision estimates (%CV) for all samples in the panel were ≤5.4%.

Simplexa Inter-lot Reproducibility – FLU A[1]

Inter-lot				
Sample ID	**n**	**Mean Ct**	**Inter-lot SD**	**Inter-lot %CV**
No Template Control	9	40.0	0.00	0.0
Positive Control	9	30.4	0.24	0.8
Flu A High Negative	9	40.0	0.00	0.0
Flu A Low Positive	9	33.3	0.06	0.2
Flu A Medium Positive	9	30.3	0.08	0.3

Simplexa Inter-lot Reproducibility – FLU B[1]

Inter-lot				
Sample ID	**n**	**Mean Ct**	**Inter-lot SD**	**Inter-lot %CV**
No Template Control	9	40.0	0.00	0.0
Positive Control	9	28.2	0.16	0.6
Flu B High Negative	9	40.0	0.00	0.0
Flu B Low Positive	9	33.4	0.39	1.2
Flu B Medium Positive	9	29.9	0.14	0.5

Simplexa Inter-lot Reproducibility – RSV[1]

Inter-lot				
Sample ID	**n**	**Mean Ct**	**Inter-lot SD**	**Inter-lot %CV**
No Template Control	9	40.0	0.00	0.0
Positive Control	9	30.3	1.65	5.4
RSV High Negative	9	40.0	0.00	0.0
RSV Low Positive	9	33.7	0.00	0.0
RSV Medium Positive	9	30.3	0.27	0.9

[1] For the purposes of calculating averages and variance components, samples that offered a negative result (Ct=0) were assigned to a value of 40.0, as a value of 40.0 is more representative of negative samples which have Ct values at the upper limit of the range.

Inter-laboratory, Inter-assay and Intra-assay Reproducibility

Sample pools of influenza A, influenza B, and RSV were created by spiking concentrated stock of each virus into a matrix generated from negative swabs. Three sample pools were generated per virus corresponding to high negative, low positive, and medium positive levels of virus. Each sample was tested in triplicate at three separate sites: one run per operator, per day, two operators per site, at three sites for 5 days. Nine sample pools corresponding to the three viruses, each at the three levels described earlier, were tested along with No Template Control and Positive Control. No Template Control and Positive Control were also tested in triplicate. A total of 990 samples were tested in the reproducibility studies. Estimates of variability were determined with a random effects model that includes run and site as predictors, with run nested within site. This allowed for partitioning of the total variance to provide best estimates of inter-assay, intra-assay, and inter-lab variability.

Three sites assessed the device's inter-laboratory reproducibility and inter/intra-assay reproducibility. Site 1 performed the extraction using the MagNA Pure LC Total Nucleic Acid Isolation Kit and Sites 2 and 3 performed the extraction step using the NucliSENS® easyMAG™ System (bioMérieux). All sample results were included in the analysis. The total %CV at each site for positive samples ranged from 0.6% to 2.1% for FLU A, from 0.5% to 6.7% for FLU B, and from 2.0% to 4.5% for RSV

Reproducibility – FLU A

Sample	Site 1			Site 2			Site 3				
	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Total Agreement with expected results	95% CI
No Template Control	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Positive Control	30/30	30.2	1.6	30/30	29.6	1.5	30/30	29.8	1.3	90/90 (100.0%)	95.9% - 100.0%
Flu A High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu A Low Positive	30/30	33.0	0.9	30/30	33.3	1.3	30/30	33.5	2.1	90/90 (100.0%)	95.9% - 100.0%
Flu A Medium Positive	30/30	29.6	0.9	30/30	29.4	0.6	30/30	29.5	0.8	90/90 (100.0%)	95.9% - 100.0%
Flu B High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu B Low Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu B Medium Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV Low Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV Medium Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Total Agreement	330/330 (100.0%)			330/330 (100.0%)			330/330 (100.0%)			990/990 (100.0%)	99.6% – 100.0%

Reproducibility – FLU B

Sample	Site 1			Site 2			Site 3				
	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Total Agreement with expected results	95% CI
No Template Control	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Positive Control	30/30	28.8	1.2	30/30	28.4	1.0	30/30	28.1	1.5	90/90 (100.0%)	95.9% - 100.0%
Flu A High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu A Low Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu A Medium Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu B High Negative	30/30	40.0	0.0	29/30	39.2	11.7	30/30	40.0	0.0	89/90 (98.9%)	94.0% - 100.0%
Flu B Low Positive	30/30	33.8	1.2	26/30	35.3	5.7	25/30	35.0	6.7	81/90 (90.0%)	82.1% - 100.0%
Flu B Medium Positive	30/30	30.4	0.7	30/30	30.1	0.5	30/30	29.9	1.2	90/90 (100.0%)	95.9% - 100.0%
RSV High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV Low Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV Medium Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Total Agreement	330/330 (100.0%)			325/330 (98.5%)			325/330 (98.5%)			980/990 (99.0%)	98.2% - 99.5%

Reproducibility – RSV

Sample	Site 1			Site 2			Site 3				
	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Total Agreement with expected results	95% CI
No Template Control	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%

Sample	Site 1			Site 2			Site 3				
	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Agreement with expected results	Avg. Ct	Total %CV	Total Agreement with expected results	95% CI
Positive Control	30/30	31.1	2.9	30/30	29.4	3.2	30/30	28.4	2.7	90/90 (100.0%)	95.9% - 100.0%
Flu A High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu A Low Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu A Medium Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu B High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu B Low Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
Flu B Medium Positive	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV High Negative	30/30	40.0	0.0	30/30	40.0	0.0	30/30	40.0	0.0	90/90 (100.0%)	95.9% - 100.0%
RSV Low Positive	30/30	33.8	4.5	30/30	33.3	3.6	30/30	32.3	2.0	90/90 (100.0%)	95.9% - 100.0%
RSV Medium Positive	30/30	30.4	4.3	30/30	28.6	4.4	30/30	28.5	4.1	90/90 (100.0%)	95.9% - 100.0%
Total Agreement	330/330 (100.0%)			330/330 (100.0%)			330/330 (100.0%)			990/990 (100.0%)	99.6% – 100.0%

b. Linearity/assay reportable range:

Not applicable.

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

Controls

The following controls are provided in the Focus Diagnostics Simplexa™ Flu A/B & RSV assay kit:

Positive Control (PC): The kit contains a Positive Control consisting of a mixture of inactivated influenza A virus, inactivated influenza B virus, and inactivated RSV. The PC, in conjunction with the Simplexa™ RNA Internal Control, is used to verify reagent and system performance. The Positive Control is meant to be a control for global failure of the assay (missing reaction component, instrument failure, etc.). A Positive Control should be included in each run.

RNA Internal Control (RNA IC): The RNA IC is an encapsulated RNA sequence. The RNA IC is incorporated into every sample and is carried through all steps of the procedure from nucleic acid isolation and purification through amplification. The RNA IC is intended to monitor for PCR inhibition.

No Template Control (NTC): The NTC consists of nuclease-free water and, after addition of RNA IC, is taken through the RNA extraction process and

subsequent amplification and detection. The NTC reaction should not exhibit fluorescence growth curves that cross the threshold line in any of the FLU A, FLU B, or RSV detection channels but must provide a valid Ct value (Ct ≤40, ≠0) for the RNA IC. If any of the FLU A, FLU B, or RSV channels provide a Ct value ≤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.

Control	Flu A Ct	Flu B Ct	RSV Ct	RNA IC Ct
No Template Control	0	0	0	≤40, ≠0
Positive Control	≤40, ≠0	≤40, ≠0	≤40, ≠0	Not Applicable (N/A)

d. Detection limits:

Analytical sensitivity was estimated for two strains of influenza A (A/PR/8/34 H1N1, A/Hong Kong/8/68 H3N2), two strains of influenza B (B/Great Lakes/1739/54, B/Malaysia/2506/2004), and two strains of RSV (RSV A2, RSV B2). The Limit of Detection (LoD) for each strain of virus was determined by limiting dilution studies using quantified stocks of the viruses. The viruses used in this study were grown, re-titered, and diluted with negative swab matrix. A preliminary estimate of the LoD for each virus was carried out by testing four dilutions of each virus around the theoretical LoD with each dilution extracted three times using the Roche MagNA Pure LC System. A single extraction of Positive Control (PC) and No Template Control (NTC) were included in each extraction cartridge. Each extracted sample and control was assayed in a single well. The lowest concentration at which all three replicates were positive were taken as the tentative LoD.

Confirmation of LoD was determined over multiple runs. Each strain was spiked into negative swab at the level of the preliminary LoD and extracted using both the Roche MagNA Pure LC System and the NucliSENS easyMAG extraction method. A single extraction of Positive Control (PC) and No Template Control (NTC) was included in each run.

The LoD was determined as the lowest concentration that was detected ≥95% of the time (i.e. concentration at which at least 19 out of 20 replicates were determined to be positive). The tables below summarize the data obtained during estimation of the preliminary LoD and confirmation of LoD for the six viral strains.

Simplexa™ FLU A/B &RSV Limit of Detection Screening and Confirmation

– FLU A

Influenza A Strain	$TCID_{50}$/mL	Initial Screening: MagNA Pure Extraction	Confirmation of LoD MagNA Pure Extraction	Confirmation of LoD NucliSENS easyMAG Extraction
A/PR/8/34 (H1N1)	1.0×10^{2}	3/3	-	-
	1.0×10^{1}	3/3	-	-
	1.0×10^{0}	3/3	-	-
	1.0×10^{-1}	3/3	-	-
	1.0×10^{-2}	3/3	20/20	20/20
A/Hong Kong/8/68 (H3N2)	1.0×10^{2}	3/3	-	-
	1.0×10^{1}	3/3	20/20	19/19
	1.0×10^{0}	2/3	-	-
	1.0×10^{-1}	0/3	-	-
	1.0×10^{-2}	0/3	-	-

Simplexa™ FLU A/B &RSV Limit of Detection – FLU A

Viral Strain	**LoD MagNA Pure extraction ($TCID_{50}$/mL)**	**LoD NucliSENS easyMAG extraction ($TCID_{50}$/mL)**
Influenza A/PR/8/34 (H1N1)	1.0×10^{-2}	1.0×10^{-2}
Influenza A/Hong Kong/8/68 (H3N2)	1.0×10^{1}	1.0×10^{1}

Simplexa™ FLU A/B &RSV Limit of Detection Screening and Confirmation – FLU B

Influenza B Strain	$TCID_{50}$/mL	Initial Screening: MagNA Pure Extraction	Confirmation of LoD MagNA Pure Extraction	Confirmation of LoD NucliSENS easyMAG Extraction
B/Great Lakes/1739/54	1.0×10^{2}	3/3	-	-
	5.0×10^{1}	3/3	-	-
	2.5×10^{1}	3/3	-	-
	1.0×10^{1}	3/3	-	-
	5.0×10^{0}	n/a	-	20/20
	1.0×10^{0}	3/3	20/20	17/20
B/Malaysia/2506/2004	1.0×10^{2}	3/3	-	-
	5.0×10^{1}	3/3	-	-
	2.5×10^{1}	3/3	-	-
	1.0×10^{1}	3/3	20/20	20/20
	1.0×10^{0}	2/3	-	-

Simplexa™ FLU A/B &RSV Limit of Detection – FLU B

Viral Strain	LoD MagNA Pure extraction ($TCID_{50}$/mL)	LoD NucliSENS easyMAG extraction ($TCID_{50}$/mL)
Influenza B/Great Lakes/1739/54	1.0×10^{0}	5.0×10^{0}
Influenza B/Malaysia/2506/2004	1.0×10^{1}	1.0×10^{1}

Simplexa™ FLU A/B &RSV Limit of Detection Screening and Confirmation – RSV

RSV Strain	$TCID_{50}$/mL	Initial Screening: MagNA Pure Extraction	Confirmation of LoD MagNA Pure Extraction	Confirmation of LoD NucliSENS easyMAG Extraction
RSV A2	1.0×10^{2}	3/3	-	-
	1.0×10^{1}	3/3	-	-
	1.0×10^{0}	3/3	20/20	20/20
	1.0×10^{-1}	2/3	-	-
	1.0×10^{-2}	0/3	-	-
RSV B1	1.0×10^{2}	3/3	-	-
	1.0×10^{1}	3/3	-	-
	5.0×10^{0}	-		20/20
	1.0×10^{0}	3/3	19/20	15/20
	1.0×10^{-1}	0/3	-	-
	1.0×10^{-2}	0/3	-	-

Simplexa™ FLU A/B &RSV Limit of Detection – RSV

Viral Strain	LoD MagNA Pure extraction ($TCID_{50}$/mL)	LoD NucliSENS easyMAG extraction ($TCID_{50}$/mL)
RSV A2	1.0×10^{0}	1.0×10^{0}
RSV B1	1.0×10^{0}	5.0×10^{0}

e. Analytical specificity:

Cross reactivity

A panel of thirty-two (32) potentially cross reacting microorganisms representing common respiratory pathogens or flora commonly present in the nasopharynx was evaluated for cross reactivity. Each potential cross reactant was individually spiked at a high level into a negative swab matrix. The level of each microorganism was determined by growing and titering the microorganism listed. The unspiked matrix was also tested to serve as a baseline. Samples were extracted with the Roche MagNA Pure LC System and tested in triplicate to screen for cross reactivity. According to the study

protocol, if influenza A virus, influenza B virus, or RSV was detected in any of the three replicates, an additional 5 replicates were to be tested for confirmation. One extraction was used to make the original three replicates and the confirmatory five replicates.

No confirmatory testing was required as all initial replicates gave negative results for influenza A, influenza B, and RSV. The results are summarized in the table below.

Simplexa Flu™ A/B & RSV – Cross Reactivity

Cross-Reactant	Testing Concentration	Units	Flu A	Flu B	RSV
Adenovirus 1	1.02 x10^5	$TCID_{50}$/mL	–	–	–
Adenovirus 7A	4.57 x10^5	$TCID_{50}$/mL	–	–	–
Bordetella pertussis	5.80 x10^5	cfu/mL	–	–	–
Chlamydia pneumoniae	1.52 x10^5	IFU/mL	–	–	–
Coronavirus 229E	2.45 x10^5	$TCID_{50}$/mL	–	–	–
Coronavirus OC43	1.70 x10^5	$TCID_{50}$/mL	–	–	
Corynebacterium diphtheriae	2.87 x10^6	cfu/mL	–	–	–
Cytomegalovirus	3.55 x10^5	$TCID_{50}$/mL	–	–	–
Enterovirus 71	1.41 x10^5	$TCID_{50}$/mL	–	–	–
Epstein Barr Virus	6.04 x10^5	copies/mL[1]	–	–	–
Escherichia coli, O157H7	2.34 x10^6	cfu/mL	–	–	–
Haemophilus influenzae	2.60 x10^6	cfu/mL	–	–	–
Lactobacillus plantarum, 17-5	1.75 x10^6	cfu/mL	–	–	–
Legionella longbeachae	7.10 x10^6	cfu/mL	–	–	–
Measles	1.26 x10^5	$TCID_{50}$/mL	–	–	–
Metapneumovirus	5.01 x10^5	$TCID_{50}$/mL	–	–	–
Moraxella catarrhalis, Ne 11	6.83 x10^6	cfu/mL	–	–	–
Mumps	8.51 x10^5	$TCID_{50}$/mL	–	–	–
Mycobacterium tuberculosis	2.20 x10^6	cfu/mL	–	–	–
Mycoplasma pneumoniae, Strain M129	5.63 x10^6	$TCID_{50}$/mL	–	–	–
Neisseria elongata	1.99 x10^6	cfu/mL	–	–	–
Neisseria meningitides	1.63 x10^6	cfu/mL	–	–	–
Parainfluenza 1	6.61 x10^5	$TCID_{50}$/mL	–	–	–
Parainfluenza 2	5.89 x10^5	$TCID_{50}$/mL	–	–	–
Parainfluenza 3	6.61 x10^5	$TCID_{50}$/mL	–	–	–
Pseudomonas aeruginosa	1.05 x10^6	cfu/mL	–	–	–
Rhinovirus 1A	3.16 x10^5	$TCID_{50}$/mL	–	–	–
Staphylcoccus aureus, COL	8.40 x10^6	cfu/mL	–	–	–
Staphylococcus epidermidis	3.80 x10^6	cfu/mL	–	–	–
Streptococcus pneumoniae	5.54 x10^6	cfu/mL	–	–	–
Streptococcus pyogenes	1.55 x10^6	cfu/mL	–	–	–
Streptococcus salivarius	1.14 x10^6	cfu/mL	–	–	–

1) The EBV virus is grown in a transformed cell line (marmoset leukocytes). Transformed cells are not an appropriate cell line for quantitation using $TCID_{50}$/mL, instead, copies/mL is calculated using a quantitative PCR method.

Reactivity

In addition to the six viral strains tested for LoD, ten additional influenza A strains, nine additional influenza B strains, and four additional strains of RSV were tested for reactivity with the Simplexa™ Flu A/B & RSV assay. A strain of 2009 H1N1 influenza A virus was included in the study. Each virus tested for reactivity was diluted in negative swab matrix at 1.0 x 10^2 $TCID_{50}$/mL and extracted using the Roche MagNA Pure LC instrument. Each strain was extracted in triplicate and assayed 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.

Analytical Reactivity with Additional Viral Strains

Viral Strain	Lowest Concentration Detected ($TCID_{50}$/mL)	Result
Influenza A/Wisconsin/67/05 H3	1.0 x10^2	Flu A Detected (3/3)
Influenza A/New Caledonia/10/07 H1N1	1.0 x10^2	Flu A Detected (3/3)
Influenza A/Brisbane/10/07 H3	1.0 x10^2	Flu A Detected (3/3)
Influenza A/Solomon Island/03/06 H1	1.0 x10^2	Flu A Detected (3/3)
Influenza A/Taiwan/42/06 H1N1	1.0 x10^2	Flu A Detected (3/3)
Influenza A/Brisbane/59/07 H1	1.0 x10^2	Flu A Detected (3/3)
Influenza A/Swine NY/02/2009 H1	1.0 x10^2	Flu A Detected (3/3)
Influenza A/WS/33 H1N1	1.0 x10^2	Flu A Detected (3/3)
Influenza A/Port Chalmers/1/73 H3N2	1.0 x10^2	Flu A Detected (3/3)
Influenza A/California/7/2009 NYMC X-179A	1.0 x10^2	Flu A Detected (3/3)
Influenza B/Florida/02/06	1.0 x10^2	Flu B Detected (3/3)
Influenza B/Florida/04/06	2.0 x10^2	Flu B Detected (3/3)
Influenza B/Florida/07/04	1.0 x10^2	Flu B Detected (3/3)
Influenza B/Lee/40	1.0 x10^2	Flu B Detected (3/3)
Influenza B/Maryland/1/59	1.0 x10^2	Flu B Detected (3/3)
Influenza B/Hong Kong/5/72	1.0 x10^2	Flu B Detected (3/3)
Influenza B/Allen/45	1.0 x10^2	Flu B Detected (3/3)
Influenza B/Taiwan/2/62	2.0 x10^2	Flu B Detected (3/3)
Influenza B/Panama/45/90	2.0 x10^2	Flu B Detected (3/3)
RSV A-Long	1.0 x10^2	RSV Detected (3/3)
RSV B-Wash/18537/62	1.0 x10^2	RSV Detected (3/3)
RSV B-WV/14617/85	1.0 x10^2	RSV Detected (3/3)
RSV B-9320	1.0 x10^2	RSV Detected (3/3)

f. Interference Studies:

Interfering Substances

The performance of the Simplexa™ Flu A/B & RSV assay was evaluated with potentially interfering substances that may be present in nasopharyngeal swab specimens at the concentrations indicated in the table below. The potentially interfering substances were evaluated in influenza A (influenza A/Hong

Kong/8/68) and influenza B (influenza B/Malaysia/2506/2004), each at a level of 50 $TCID_{50}$/mL, and RSV A2 at a level of 5 $TCID_{50}$/mL. All samples of virus plus potential interferent were extracted with the Roche MagNA Pure LC instrument and tested in triplicate. There was no evidence of interference caused by the substances tested.

Interfering Substances Panel

Potential Interferents	Active Ingredient	Interferent Concentration
Afrin Nasal Spray	Oxymetazoline	15% (v/v)
Anti-viral drug-Relenza	Zanamivir	3.3 mg/ml
Anti-viral drug-Tamiflu	Oseltamivir	25 mg/ml
Antibacterial, systemic	Tobramycin	4.0 μg/ml
Antibiotic, nasal ointment	Mupirocin	6.6 mg/ml
Blood	N/A	2% (v/v)
Mucin Bovine Submaxillary Glad Type I-S	Purified Mucin Protein	60 μg/ml
Nasal Corticosteroid-Beconase AQ	Beclomethasone	5% (v/v)
Nasal Corticosteroid-Fluticasone	Fluticasone	5% (v/v)
Zicam Nasal Gel	Luffa Opperculata, Galphimia glauca, histaminum hydrochloricum	5% (v/v)

Interfering Microorganisms

A study was carried out to determine if any of the panel of thirty-two potentially cross-reacting microorganisms (listed in Section M.1.e (*Analytical specificity*)) could inhibit detection of influenza A, influenza B, or RSV by the Simplexa™ Flu A/B & RSV assay. These potentially inhibitory microorganismsms were individually spiked into a pool containing a low level (2 x LoD) of influenza A (A/PR/8/34), influenza B (B/Malaysia/2506/2004) and RSV (A2). Each sample was tested in triplicate. The results from the study are summarized in the table below. No inhibitory effects on detection of influenza A, influenza B, or RSV were confirmed.

Microorganism	Microorganism Concentration	Concentration Units	Flu A	Flu B	RSV
Adenovirus 1	1.1×10^5	$TCID_{50}$/mL	+	+	+
Adenovirus 7A	1.1×10^5	$TCID_{50}$/mL	+	+	+
Bordatella pertussis	1.1×10^6	cfu/mL	+	+	+
Chlamydia pneumoniae	1.1×10^6	copies/mL	+	+	+
CMV	1.1×10^5	$TCID_{50}$/mL	+	+	+

Microorganism	Microorganism Concentration	Concentration Units	Flu A	Flu B	RSV
Coronavirus 229E	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Coronavirus OC43[1]	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Corynebacterium diphtheriae	1.1 x10^6	cfu/mL	+	+	+
E. coli O157	1.1 x10^6	cfu/mL	+	+	+
EBV	1.1 x10^5	copies/mL	+	+	+
Enterovirus 71	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Haemophilus influenzae	1.1 x10^6	cfu/mL	+	+	+
Lactobacillus plantarum, 17-5	1.1 x10^6	cfu/mL	+	+	+
Legionella longbeachae	1.1 x10^6	cfu/mL	+	+	+
Measles	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Metapneumovirus[1]	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Moraxella catarrhalis Ne11	1.1 x10^6	cfu/mL	+	+	+
Mumps	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Mycobacterium tuberculosis	1.1 x10^6	cfu/mL	+	+	+
Mycoplasma pneumoniae M129	1.1 x10^6	$TCID_{50}$/mL	+	+	+
Neisseria elongata	1.1 x10^6	cfu/mL	+	+	+
Neisseria meningitides	1.1 x10^6	cfu/mL	+	+	+
Parainfluenza 1	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Parainfluenza 2	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Parainfluenza 3	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Pseudomonas aeruginosa	1.1 x10^6	cfu/mL	+	+	+
Rhinovirus 1A	1.1 x10^5	$TCID_{50}$/mL	+	+	+
Staphylococcus aereus, COL	1.1 x10^6	cfu/mL	+	+	+
Staphylococcus epidermidis	1.1 x10^6	cfu/mL	+	+	+
Streptococcus pneumoniae	1.1 x10^6	cfu/mL	+	+	+
Streptococcus pyogenes	1.1 x10^6	cfu/mL	+	+	+
Streptococcus salivarius	1.1 x10^6	cfu/mL	+	+	+

[1] Initial testing appeared to show possible inhibition, upon repeat testing there was no evidence of inhibition.

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™ FluA/B &RSV assay performance for another target present at low levels in the multiplex assay. A low positive sample was contrived at approximately 4 to 6 times the LoD for each target (influenza A, influenza B and RSV), and a baseline Ct was determined for each sample. A high level of each potential concomitant infecting virus was spiked into the low level specimen as summarized in the table below.

Baseline Strain (Concentration)	Competitive Strain (Concentration)

Influenza A/PR/8/34 (8.80×10^1 $TCID_{50}$/mL)	RSV A2 (1.60×10^4 $TCID_{50}$/mL)
Influenza B/Malaysia/2506/2004 (1.29×10^2 $TCID_{50}$/mL)	RSV A2 (1.60×10^4 $TCID_{50}$/mL)
RSV A2 (<10 $TCID_{50}$/mL)	Influenza A/PR/8/34 (2.50×10^5 $TCID_{50}$/mL)*
	Influenza B/Malaysia/2506/2004 (2.58×10^5 $TCID_{50}$/mL)

*When very high levels of influenza A are present with low levels of RSV the signal from the RSV reaction may not be adequate to be detected, due to competitive interference. When all three viruses are present at moderate levels (as in the Positive Control) there is no evidence of competitive interference.

g. Assay cut-off:

Forty-five cycles of amplification were performed during assay development to allow for the appropriate determination of assay cut off. Analysis of the Limit of Detection study showed that all Ct values at the Limit of Detection were <40 with the exception of one influenza A sample which gave a Ct result >40. The Limit of Detection is defined as the lowest concentration of sample where ≥ 95% of twenty replicates are detected. A review of the Method Comparison (Clinical Performance) data showed that >95% of the positive clinical specimens had a Ct value ≤35. Specimens that were positive for influenza A had Ct values in the range 15.2 to 37.3. Specimens that were positive for influenza B had Ct values in the range 16.5 to 34.0. Specimens that were positive for RSV had Ct values in the range 16.0 to 41.4 with one specimen giving a Ct value >40. The data thus indicate that Ct=40 is an appropriate assay cut-off

h. Carry-over/Cross-Contamination

The study was designed by alternately placing high positive and high negative (5×10^6 dilution of high positive) samples on both the MagNA Pure cartridge for extraction and the Universal Disc for amplification. A total of 180 high negative samples were tested across 5 runs. The sample size of 180 was chosen to provide 80% power of detecting a carry over/contamination effect of 5% or greater, with alpha = 0.05. The carryover effect was evaluated by comparing the observed positive rate for the high negative samples with the expected rate under normal reproducibility conditions. The purpose of analysis was to determine if the positive rate for the high negative samples was elevated beyond what was probable. A one sample, one sided test for a proportion (binomial) was used to test for significance for all three FLU A, FLU B, and RSV channels.

The baseline negative samples were positive 0% (0 of 90 replicates) of the time for the FLU B and RSV channels and 1.1% (1of 90 replicates) of the time for the FLUA channel. When tested with high positive samples, no significant carry-over contamination effect was seen in any of the channels. The data from the study are summarized in the table below.

Carry-Over Contamination

	Channel		
	FLU A	FLU B	RSV
Positive Count	2	0	0
Negative Count	178	180	180
Total observations2	180	180	180
Percent positive	1.1	0	0
One-sided binomial test (p-value)	0.0054	0.00	0.00

2. Comparison studies:

   a. *Method comparison with reference methods:*

   The performance of the Simplexa™ FluA/B &RSV assay was compared with reference methods during testing of clinical specimens. For influenza A and influenza B, the reference method was a high performance FDA cleared nucleic acid test. For RSV, the reference method was culture/DFA.

   b. *Matrix Comparison:*

   Not applicable

   c. *Performance in Fresh vs. Frozen Clinical Specimens:*

   The performance of the Simplexa™ FluA/B &RSV assay was determined by comparing fresh (never frozen, unextracted and tested within 72 hours of collection) versus frozen (unextracted) clinical specimens. A panel of one hundred and eighty (180) nasopharyngeal swab specimens including sixty (60) positive swabs for each virus (influenza A, influenza B, RSV) was contrived for testing so that they covered the range expected from clinical specimens. Three aliquots of each sample were prepared, representing fresh, short-term frozen (at least 2 hours frozen at -20ºC) and long-term frozen (at least 7 days frozen at -70ºC) states. All testing was performed at Focus Diagnostics using one Simplexa ™ Flu A/B & RSV lot.

   The plots of short-term frozen Ct values and long-term frozen Ct values vs. fresh Ct values visually show the existence of a linear relationship. Additionally, for all comparisons, the value of the coefficient of determination (R^2) is greater than 0.95, meaning that at least 95% of the variation seen in the frozen (short-term and long-term) Ct values are explained by the variation seen in fresh Ct values.
   The slopes, along with the 95% confidence interval and percent bias for the above comparisons, are summarized in the following table. The 95% confidence interval indicates that slope estimates are not statistically significant at 5% level of significance. The observed bias for both short-term and long-term frozen Ct values is small and is not clinically significant. This

indicates that short-term and long-term frozen samples are stable in comparison with fresh samples.

Channel	Frozen Term	Slope	95% Confidence Interval	R2	Bias
FLU A[1]	Short-term	1.00	0.997 – 1.001	1.00	0% - 3.0%
	Long-term	1.00	1.001 – 1.006	1.00	0%
FLU B[1]	Short-term	1.00	0.993 – 1.003	1.00	0%
	Long-term	1.03	1.020 – 1.030	1.00	3%
RSV[1]	Short-term	1.04	1.011 – 1.076	0.96	4%
	Long-term	1.06	1.023 – 1.097	0.95	1.0%6%

[1] For the purposes of regression analysis, samples that offered a negative result (Ct=0) were assigned to a value of 40.0, as a value of 40.0 is more representative of negative samples which have Ct values at the upper limit of the range.

d. Extraction Efficiency:

Assessment of the equivalence of the two extraction methods was determined by several approaches: (1) One clinical testing site performed Clinical Performance and Reproducibility studies using the MagNA Pure LC Total Nucleic Acid Isolation kit and two sites used the NucliSENS easyMAg system and (2) Determinations of Limit of Detection were carried out using both extraction systems. Based on the results from these studies, there does not appear to be any significant difference in the performance of the Simplexa™ FluA/B &RSV assay using either the MagNA Pure LC Total Nucleic Acid Isolation kit or the NucliSENS easyMAg system.

3. Clinical studies:

Three external testing sites participated in the Clinical Agreement Study. Reference results for influenza A and influenza B viruses were generated using a high performance FDA cleared nucleic acid test (NAT). Reference results for RSV were generated using culture/DFA. Culture/DFA results were carried forward from the results obtained at the time of sample collection or banking. A total of 735 nasopharyngeal swabs specimens were obtained from a combination of prospectively collected specimens (n = 558) and retrospective, banked specimens (n=177) from patients with signs and symptoms of viral respiratory tract infection.

Prospective samples were collected in Southern United States from 26-February-2010 to 24-March-2010 and in Australia from 04-July-2010 to 17-August-2010. Three (3) samples were excluded from the prospective analysis due to "Unresolved" status on the reference nucleic acid assay and two (2) samples were excluded due to "invalid" status on the Simplexa assay. The samples remained unresolved or invalid upon repeat testing. One (1) sample was excluded from the prospective analysis due to lack of culture/DFA result for RSV. Due to the low prevalence rate of influenza during the collection period and the rareness of RSV in the adult population, retrospectively collected specimens from the, 2008 – 2009

and early 2010 influenza and RSV seasons in Eastern and Mid-Western United States from patients with signs and symptoms of viral respiratory tract infection were also evaluated (n = 177); of the 177 samples collected 47 were not analyzed by culture/DFA for influenza A or influenza B.

The clinical samples were tested at three sites. One site extracted specimens using the MagNA Pure LC Total Nucleic Acid Isolation kit and the other two sites used the NucliSENS easyMAg system. No apparent different difference in clinical performance was observed for the two extraction systems. The clinical performance of the Simplexa™ FluA/B &RSV assay is summarized in the following tables.

Influenza A Clinical Summary – Prospective Samples

*High Perf. NAT[1]		Simplexa		
	n	Detected	Not Detected	% Agreement
Detected	25	25	0	100%(25/25) 95% CI:86.7-100%
Not Detected	528	1	527	99.8%(527/528) 95% CI:98.9-100%

(Culture/DFA)		Simplexa		
	n	Detected	Not Detected	Sensitivity/Specificity
Detected	22	22	0	100%(22/22) 95% CI:85.1-100%
Not Detected	534	4	530	99.3%(530/534) 95% CI:98.1-99.7%

Influenza B Clinical Summary – Prospective Samples

*High Perf. NAT[1]		Simplexa		
	n	Detected	Not Detected	% Agreement
Detected	2	1	1	50%(1/2) 95% CI:9.5-90.5%
Not Detected	551	1	550	99.8%(550/551) 95% CI:99-100%

(Culture/DFA)		Simplexa		
	n	Detected	Not Detected	Sensitivity/Specificity
Detected	1	1	0	100%(1/1) 95% CI:20.7-100%
Not Detected	555	1	554	99.8%(554/555) 95% CI:99-100%

RSV Clinical Summary – Prospective Samples

NAT		Simplexa		
	n	Detected	Not Detected	% Agreement
Detected	111	110	1	99.1%(110/111) 95% CI:95.1-99.8%
Not Detected	442	2	440	99.5%(440/442) 95% CI:98.4-99.9%

(Culture/DFA)		Simplexa		
	n	Detected	Not Detected	Sensitivity/Specificity
Detected	100	98	2	98%(98/100) 95% CI:93-99.4%
Not Detected	455	14	441	96.9%(441/455) 95% CI:94.9-98.2%

Influenza A Clinical Agreement Summary – Retrospective Samples

*High Perf. NAT[1]		Simplexa		
	n	Detected	Not Detected	% Agreement
Detected	79	79	0	100%(79/79) 95% CI:95.4-100%
Not Detected	98	1	97	99%(97/98) 95% CI:94.4-99.8%

(Culture/DFA)		Simplexa		
	n	Detected	Not Detected	Sensitivity/Specificity
Detected	80	80	0	100%(80/80) 95% CI:95.4-100%
Not Detected	50	0	50	100%(50/50) 95% CI:92.9-100%

Influenza B Clinical Agreement Summary – Retrospective Samples

*High Perf. NAT[1]		Simplexa		
	n	Detected	Not Detected	% Agreement
Detected	50	50	0	100%(50/50) 95% CI:92.9-100%
Not Detected	127	0	127	100%(127/127) 95% CI:97.1-100%

(Culture/DFA)		Simplexa		
	n	Detected	Not Detected	Sensitivity/Specificity
Detected	50	50	0	100%(50/50) 95% CI:92.9-100%
Not Detected	93	0	93	100%(93/93) 95% CI:96-100%

RSV Clinical Agreement Summary – Retrospective Samples

NAT		Simplexa		
	n	Detected	Not Detected	% Agreement
Detected	22	22	0	100%(22/22) 95% CI:85.1-100%
Not Detected	155	1	154	99.4%(154/155) 95% CI:96.4-99.9%

(Culture/DFA)		Simplexa		
	n	Detected	Not Detected	Sensitivity/Specificity
Detected	22	22	0	100%(22/22) 95% CI:85.1-100%
Not Detected	25	1	24	96%(24/25) 95% CI:80.5-99.3%

[1]High Perf. NAT = High Performance FDA Cleared Nucleic Acid Test
*Reference method for clinical performance evaluation for 510(k) clearance.

4. Clinical cut-off**:** N/A

5. Expected values/Reference range:

Prospective specimens used in this clinical study were obtained from the United States (Texas and Ohio) and Australia. The prevalence of all influenza viruses in the US[1] during the February to March 2010 collection period ranged from 3.5 to 6.4%. Among influenza positives, 97 to 99.6% were positive for Influenza A and 0 to 1.5% for Influenza B.

Prevalence observed during the clinical study

Region	Flu A	Flu B	RSV
Texas[2]	10.2%	0%	18.0
Ohio[2]	0%	1.0%	49.0%
Australia[3]	1.6%	0.4%	5.6%

1. http://www.cdc.gov/flu/weekly/fluactivity.htm
2. Collection period was February to March, 2010.
3. Collection period was July to August, 2010.

N. Instrument Name:

Integrated Cycler with Integrated Cycler Studio Software version 2.1 or higher (3M)

MagNA Pure LC Instrument (Roche)

NucliSENS® easyMAG™ System (bioMérieux)

O. System Descriptions:

1. Modes of Operation:

The Microfluidic Molecular 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 disc can process up to 96 independent samples.

The Roche MagNA Pure LC is an automated nucleic acid isolation and purification system based upon binding of nucleic acids to glass particles and has the capability to process a total of 32 reactions within one run. Nucleic acid is purified in multiple plastic reaction tips and cartridges by several steps that include cell lysis and binding of nucleic acid to magnetic glass particles, wash steps, and a heated elution to unbind the nucleic acid from the glass particles.

The bioMérieux NucliSens easyMAG is an automated nucleic acid isolation and purification system that is based upon the same silica extraction technology as the MagNA Pure. The easyMAG is capable of processing a total of 24 reactions with variable sample types, sample volumes, and elution volumes within a single run. Nucleic acid is purified within a single cartridge by several steps that include lysis and binding of nucleic acid to high affinity magnetic silica beads, a series of wash steps and heated elution of purified nucleic acid from the silica beads.

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:

   User manually enters Patient ID/Sample ID.

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:

   Not applicable

6. Quality Control:

   The following controls are provided:

   Positive Control (PC): A mixture of inactivated influenza A virus, inactivated influenza B virus, and inactivated RSV. The PC, in conjunction with the RNA IC is used to verify reagent and system performance. The positive control is meant to be a control for global failure of the assay (missing reaction component, instrument failure, etc.). A positive control should be included in each run.

   RNA Internal Control (RNA IC): The RNA IC is an encapsulated RNA sequence. The RNA IC is incorporated into every sample and is carried through all steps of the procedure from nucleic acid isolation and purification through amplification. The RNA IC is intended to monitor for PCR inhibition..

   No Template Control (NTC): The NTC consists of nuclease-free water and, after addition of RNA IC, is taken through the RNA extraction process and subsequent amplification and detection. The NTC reaction should not exhibit fluorescence growth curves that cross the threshold line in any of the FLU A, FLU B, or RSV detection channels but must provide a valid Ct value (Ct $\leq$40, $\neq$0) for the RNA IC. If any of the FLU A, FLU B, or RSV channels provide a Ct value $\leq$40, $\neq$0 for the NTC, contamination may have occurred in one or more components of the system. The NTC should be included in each run.

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 809.1.

R. Conclusion:

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