510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K201505

B Applicant

DiaSorin Molecular LLC

C Proprietary and Established Names

Simplexa Flu A/B & RSV Direct Gen II, Simplexa Flu A/B & RSV Positive Control Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OCC	Class II	21 CFR 866.3980 - Respiratory Viral Panel Multiplex Nucleic Acid Assay	MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

The submission contains data in support of modifications made to the Simplexa Flu A/B & RSV Direct K152408 (predicate). The changes were made to improve assay sensitivity for all three targets and to decrease reagent competition within the assay.

No design or formulation changes have been made to the Positive Control Pack.

Measurand:

Viral RNA from Influenza A (Flu A), Influenza B (Flu B) and RSV

B Type of Test:

Real-time RT-PCR system, qualitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Simplexa Flu A/B & RSV Direct Gen II

The DiaSorin Molecular Simplexa™ Flu A/B & RSV Direct Gen II assay is intended for use on the LIAISON® MDX 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.

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

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

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

Simplexa™ Flu A/B & RSV Positive Control Pack

DiaSorin Molecular's 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 and the Simplexa™ Flu A/B & RSV Direct Gen II kit for use on the LIAISON® MDX instrument. This control is not intended for use with other assays or systems.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Special Instrument Requirements:

LIAISON MDX instrument with LIAISON MDX Studio Software

IV Device/System Characteristics:

A Device Description:

The system consists of the Simplexa Flu A/B & RSV Direct Gen II assay reagents (supplied in a quantity for 24 reactions), the LIAISON MDX (with LIAISON MDX Studio Software), the Direct Amplification Disc and associated accessories. The LIAISON MDX instrument is a real-time Polymerase Chain Reaction (PCR) thermocycler used for the identification of nucleic acids from biological specimens. The Direct Amplification Disc (DAD) is compartmentalized into eight separate wedges and up to eight separate specimens or controls may be processed on each disc. Each wedge contains sample and reagent input wells, microfluidic channels and laser activated valves to control the fluid flow, and a reaction chamber. The user adds 50 µL of Reaction Mix to the reagent input well and 50 µL of unextracted specimen to the sample input well. The reverse transcription, amplification and detection are performed automatically by the instrument.

B Principle of Operation:

The Simplexa Flu A/B & RSV Direct Gen II assay uses bi-functional fluorescent probe-primers together with corresponding reverse primers to amplify Flu A, Flu B, RSV and internal control RNA. The assay targets conserved regions of influenza A viruses (matrix gene), influenza B viruses (matrix gene) and RSV (M gene) to identify these viruses in direct nasopharyngeal swabs.

The LIAISON MDX uses fluorescence to quantify the amount of targeted nucleic acid sequences in a sample. The LIAISON MDX has four optical detection channels, allowing for simultaneous detection of up to four targets. Each module is optimized for a specific detection dye based on its spectral characteristics. Each module contains a LED excitation source, filters, lenses and a fiber port. Fluorescence is collected at the fiber port and transmitted to a single photomultiplier detector. A sample is considered positive for a particular target if intensity of the optical reading (fluorescence) crosses a particular threshold before a predetermined cutoff cycle.

An RNA internal control is used to detect RT-PCR failure and/or inhibition.

C Instrument Description Information:

1. Instrument Name:

LIAISON MDX System

2. Specimen Identification:

   Barcode scanner or manual entry

3. Specimen Sampling and Handling:

   Uses direct nasopharyngeal specimens collected in Universal Transport Media (UTM)

4. Calibration:

   Each reagent kit comes with a barcode card, which contains assay specific parameters and lot information. The barcode card is scanned prior to each run.

5. Quality Control:

   The Simplexa™ Flu A/B & RSV Positive Control Pack may be used as an external positive control. Universal transport media (UTM) may be used as a negative external control (No Template Control).

V Substantial Equivalence Information:

A Predicate Device Name(s):

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

B Predicate 510(k) Number(s):

K152408

C Comparison with Predicate(s):

Device & Predicate Device(s):	K201505	K152408
Device Trade Name	Simplexa Flu A/B & RSV Direct Gen II and Simplexa Flu A/B & RSV Positive Control Pack	Simplexa Flu A/B & RSV Direct and Simplexa Flu A/B & RSV Positive Control Pack
General Device Characteristic Similarities		
Intended Use/Indications for Use	Simplexa™ Flu A/B & RSV Direct Gen II	Simplexa™ Flu A/B & RSV Direct

	The DiaSorin Molecular Simplexa™ Flu A/B & RSV Direct Gen II assay is intended for use on the LIAISON® MDX 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. Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009 H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to the state or local health department for testing. Viral culture should not be	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,

	attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens. <u>Simplexa™ Flu A/B & RSV Positive Control Pack,</u> The DiaSorin Molecular 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 and the Simplexa™ Flu A/B & RSV Direct Gen II kit for use on the LIAISON® MDX instrument. This control is not intended for use with other assays or systems.	specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens. <u>Simplexa™ Flu A/B & RSV Positive Control Pack</u> 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.
Instrument	LIAISON MDX	Same
Measurand	Viral RNA	Same
Organisms Detected	Influenza A, Influenza B and RSV	Same
Genomic Targets	Influenza A matrix gene, Influenza B matrix gene, RSV M gene	Same
Technology	Real-time RT-PCR	Same
Quality Controls	Simplexa Flu A/B & RSV Positive Control Pack	Same
Device Characteristics Differences		
Modifications were made to optimize the reaction mix formulation and concentrations of primer/probes and buffers. Updated cycling parameters (thresholds) and cutoff Ct values for all targets. Added Analytical Reactivity information for additional strains that can be detected with the kit.		
Catalog No. (assay)	MOL2655	MOL2650

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

The modified device was evaluated in a reproducibility study testing a panel of eight samples, representing a Low Positive (2x LoD) and a Moderate Positive (4x LoD) level for each virus. The panel members were prepared by diluting viral stocks of influenza A, influenza B and RSV strains (re-grown and titered) into pooled negative clinical matrix (NP swabs collected in UTM). The panel included a Positive Control and a negative sample consisting of UTM (no-template control).

Reproducibility Sample Panel

	Panel Member	Level	Concentration	
			$TCID_{50}$/mL	Copies/mL
1	Influenza A/Hong Kong/8/68 (H3N2)	LP (2x LoD)	4	4,858
2	Influenza A/Hong Kong/8/68 (H3N2)	MP (4x LoD)	8	9,716
3	Influenza B/Massachusetts/02/2012	LP (2x LoD)	10	1,910
4	Influenza B/Massachusetts/02/2012	MP (4x LoD)	20	3,820
5	RSV-A2	LP (2x LoD)	20	1,858
6	RSV-A2	MP (4x LoD)	40	3,716
7	UTM (No Template Control	Neg	N/A	N/A
8	Positive Control	Pos	N/A	N/A

The study was conducted at three distinct locations/work environments within the sponsor's facility to simulate three testing sites, where each site had two designated LIAISON MDX instruments and two dedicated operators. Each of the two operators used a unique lot of the reaction mix (for a total of two lots). Each panel member was tested in three replicates, in two runs per day, over five days, at three locations. A total of 90 measurements (3 replicates x 2 runs/day x 5 days x 3 sites) were generated for each panel member. The study design allowed for evaluation of multiple components of variance.

The data was assessed for (a) qualitative results, i.e., percent agreement with expected results, and (b) quantitative analysis of variance components, i.e., average Ct values and calculated SD and %CV.

Reproducibility Qualitative Results

Panel Member	Site 1	Site 2	Site 3	All Sites
	Agreement with Expected Results	Agreement with Expected Results	Agreement with Expected Results	95% CI
Flu A Low Positive	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	95.9% -100.0%

Flu A Moderate Positive	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	95.9% -100.0%
Flu B Low Positive	30/30 (100.0%)	30/30[1] (100.0%)	30/30 (100.0%)	95.9% -100.0%
Flu B Moderate Positive	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)	95.9% -100.0%
RSV (A2) Low Positive	30/30 (100%)	30/30 (100.0%)	30/30 (100.0%)	95.9% -100.0%
RSV (A2) Moderate Positive	30/30[2] (100.0%)	30/30 (100.0%)	30/30 (100.0%)	95.9% -100.0%
Positive Control	30/30 (100%)	30/30 (100.0%)	30/30 (100.0%)	95.9% -100.0%
UTM	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	95.9% -100.0%

[1]One false positive Flu A result observed when testing the Flu B Low Positive Sample
[2]One false positive Flu A result observed when testing the RSV Moderate Positive Sample

Reproducibility Quantitative Results (by Site)

Panel Member	Site 1		Site 2		Site 3		All Sites		
	Avg. Ct	Total %CV	Avg. Ct	Total %CV	Avg. Ct	Total %CV	Avg. Ct	Total %CV	95% CI
Flu A Low Positive	30.8	2.3%	31.4	1.4%	30.7	1.2%	31.0	2.0%	95.9% - 100.0%
Flu A Moderate Positive	29.8	2.5%	30.1	3.4%	29.6	1.6%	29.8	2.6%	95.9% - 100.0%
Flu B Low Positive	30.7	1.9%	30.5	3.4%	31.2	3.9%	30.8	3.3%	95.9% - 100.0%
Flu B Moderate Positive	29.8	1.4%	29.5	1.9%	29.8	2.1%	29.7	1.9%	95.9% - 100.0%
RSV (A2) Low Positive	29.7	2.3%	29.6	1.8%	29.8	2.1%	29.7	2.1%	95.9% - 100.0%
RSV (A2) Moderate Positive	29.1	1.7%	28.7	1.5%	28.7	1.5%	28.8	1.7%	95.9% - 100.0%
Positive Control	25.3	0.8%	24.4	4.7	25.0	2.9	24.9	3.4%	95.9% - 100.0%
UTM	0.0	N/A	0.0	N/A	0.0	N/A	0.0	N/A	N/A

Reproducibility Variance Components Summary

Panel Member	N	Mean Ct	Within Run		Between Day		Between Operator/ Lot		Between Site/ Instruments		Total Reproducibility	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Flu A Low Positive	90	31.0	0.50	1.6	0.00	0.0	0.21	0.7	0.34	1.1	0.64	2.1

Flu A Mod. Positive	90	29.8	0.74	2.5	0.16	0.5	0.22	0.7	0.04	0.1	0.79	2.6
Flu B Low Positive	90	30.8	0.96	3.1	0.00	0.0	0.24	0.8	0.22	0.7	1.02	3.3
Flu B Mod. Positive	90	29.7	0.48	1.6	0.00	0.0	0.29	1.0	0.00	0.0	0.56	1.9
RSV (A2) Low Positive	90	29.7	0.60	2.0	0.00	0.0	0.17	0.6	0.00	0.0	0.62	2.1
RSV (A2) Mod. Positive	90	28.8	0.46	1.6	0.00	0.0	0.00	0.0	0.22	0.8	0.51	1.8
Positive Control	90	24.9	0.61	2.5	0.50	2.0	0.23	0.9	0.33	1.3	0.88	3.6
UTM	90	0.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

The average Ct for the IC RNA, across all channels, for all samples tested, ranged from 27.6 to 28.0, with %CV range from 2.8% to 7.2%.

2. Linearity:

   Not applicable (qualitative test)

3. Analytical Specificity/Cross-reactivity

   The Simplexa Flu A/B & RSV Direct Gen II assay's analytical specificity was evaluated in a study testing samples containing organisms that are present as normal flora in the nasopharyngeal passages as well as organisms that cause clinical symptoms similar to illness with influenza A, influenza B, and/or RSV infection. Thirty-two different bacteria and viruses were included in the study, with each organism tested in three replicates. No cross reactivity was observed with the organisms at the concentrations tested, as shown below.

Analytical Cross-reactivity for Simplexa Flu A/B & RSV Direct Gen II

Organism	Tested Concentration	% Detection		
		Flu A	Flu B	RSV
Adenovirus Type 1	1×10^6 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Adenovirus Type 7A	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Bordetella pertussis A639	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Chlamydia pneumoniae	1×10^6 IFU/mL	0.0%	0.0%	0.0%
Cytomegalovirus (CMV)	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Coronavirus 229E	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Coronavirus OC43	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Corynebacterium diphtheriae	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Enterovirus Type 71	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%

Organism	Tested Concentration	% Detection		
		Flu A	Flu B	RSV
Epstein-Barr Virus (EBV)	1×10^6 copies/mL	0.0%	0.0%	0.0%
Escherichia coli O157-H7	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Haemophilus influenzae	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Lactobacillus plantarum, 17-5	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Legionella longbeachae	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Measles	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Metapneumovirus 9	1×10^6 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Moraxella catarrhalis, NE1	1×10^6 CFU/mL	0.0%	0.0%	0.0%
Mumps	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Mycobacterium tuberculosis (genomic DNA)	1×10^7 copies/mL	0.0%	0.0%	0.0%
Mycoplasma pneumoniae, M129	1×10^6 CCU/mL	0.0%	0.0%	0.0%
Neisseria elongata	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Neisseria meningitidis	1×10^6 CFU/mL	0.0%	0.0%	0.0%
Parainfluenza 1	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Parainfluenza 2	1×10^6 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Parainfluenza 3	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Pseudomonas aeruginosa	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Rhinovirus 1A	1×10^5 $TCID_{50}$/mL	0.0%	0.0%	0.0%
Staphylococcus aureus, COL	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Staphylococcus epidermidis	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Streptococcus pneumoniae	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Streptococcus pyogenes, M1	1×10^7 CFU/mL	0.0%	0.0%	0.0%
Streptococcus salivarius	1×10^7 CFU/mL	0.0%	0.0%	0.0%

4. Interference

Microbial Interference

The performance of the Simplexa Flu A/B & RSV Direct Gen II assay was evaluated for its ability to accurately detect influenza A, influenza B, and RSV in the presence of other clinically relevant pathogens. The same panel as above, consisting of 32 potentially inhibitory organisms, was individually spiked into a pool containing low concentrations (approximately 2x LoD) each of the influenza A, influenza B and RSV (A2) viruses. Samples were assayed in three replicates. No inhibition was observed for influenza A, influenza B, or RSV by the organisms at the concentrations shown below.

Microbial Interference for Simplexa Flu A/B & RSV Direct Gen II

Organism	Tested Concentration	% Detection		
		Flu A	Flu B	RSV
Adenovirus Type 1	1×10^6 $TCID_{50}$/mL	100%	100%	100%
Adenovirus Type 7A	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Bordetella pertussis A639	1×10^7 CFU/mL	100%	100%	100%
Chlamydia pneumoniae	1×10^6 IFU/mL	100%	100%	100%
Cytomegalovirus (CMV)	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Coronavirus 229E	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Coronavirus OC43	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Corynebacterium diphtheriae	1×10^7 CFU/mL	100%	100%	100%
Enterovirus Type 71	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Epstein-Barr Virus (EBV)	1×10^6 copies/mL	100%	100%	100%
Escherichia coli O157-H7	1×10^7 CFU/mL	100%	100%	100%
Haemophilus influenzae	1×10^7 CFU/mL	100%	100%	100%
Lactobacillus plantarum, 17-5	1×10^7 CFU/mL	100%	100%	100%
Legionella longbeachae	1×10^7 CFU/mL	100%	100%	100%
Measles	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Metapneumovirus 9	1×10^6 $TCID_{50}$/mL	100%	100%	100%
Moraxella catarrhalis, NE1	1×10^6 CFU/mL	100%	100%	100%
Mumps	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Mycobacterium tuberculosis (genomic DNA)	1×10^7 copies/mL	100%	100%	100%
Mycoplasma pneumoniae, M129	1×10^6 CCU/mL	100%	100%	100%
Neisseria elongata	1×10^7 CFU/mL	100%	100%	100%
Neisseria meningitidis	1×10^6 CFU/mL	100%	100%	100%
Parainfluenza 1	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza 2	1×10^6 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza 3	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Pseudomonas aeruginosa	1×10^7 CFU/mL	100%	100%	100%
Rhinovirus 1A	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Staphylococcus aureus, COL	1×10^7 CFU/mL	100%	100%	100%
Staphylococcus epidermidis	1×10^7 CFU/mL	100%	100%	100%
Streptococcus pneumoniae	1×10^6 CFU/mL	100%	100%	100%
Streptococcus pyogenes, M1	1×10^7 CFU/mL	100%	100%	100%

Organism	Tested Concentration	% Detection		
		Flu A	Flu B	RSV
Streptococcus salivarius	1 x 10^7 CFU/mL	100%	100%	100%

Competitive Interference

The performance of the Simplexa Flu A/B & RSV Direct Gen II assay was evaluated for its ability to accurately detect influenza A, influenza B, and RSV in cases of co-infection with the assay's target organisms. A low-level sample (Baseline Sample) was contrived at 4x LoD for each target (influenza A, influenza B, RSV A, and RSV B), and a baseline Ct was determined for each sample, as shown below.

Baseline Sample Concentrations and Ct Values

Virus	LoD ($TCID_{50}$/mL)	Baseline Sample (4x LoD)		Baseline Ct Value	Channel
		$TCID_{50}$/mL	Copies/mL		
Inluenza A/Hong Kong/8/68	2	8	9,716	29.6	FAM
Influenza B/Massachusetts/02/2012	5	20	3,820	29.3	JOE
RSV-A2	10	40	3,716	29.3	CR610
RSV-B CH93-18(18)	20	80	25,968	28.8	CR610

Each potential concomitant infecting virus was spiked into the Baseline Samples at approximately 10,000x LoD and assayed in triplicate. The following combinations were tested.

Competitive Interference Test Panel

Sample	High Positive (4x LoD)	Low Positive (10,000x LoD)
1	Flu A	Flu B
2	Flu A	RSV A
3	Flu A	RSV B
4	Flu B	Flu A
5	Flu B	RSV A
6	Flu B	RSV B
7	RSV A	Flu A
8	RSV A	Flu B
9	RSV B	Flu A
10	RSV B	Flu B

Because the study revealed interference of Flu A, when at concentrations of 10,000x LoD, additional testing was conducted with Flu A "interferent" samples contrived at 5,000x LoD and 1,000x LoD to determine the approximate concentration where no interference was observed with Flu B and RSV. The testing results from the study are summarized below.

Competitive Interference Results for Simplexa Flu A/B & RSV Direct Gen II

Target Organism	Conc. $TCID_{50}$/mL	Baseline Avg. Ct	Competitive Strain	Conc. $TCID_{50}$/mL	No. Detected	Average Ct		
						Flu A Ct	Flu B Ct	RSV Ct
Influenza A/Hong Kong/8/68	8	29.7	Flu B	5.00×10^4	3/3	29.8	15.3	N/A
			RSV A	1.00×10^5	3/3	29.1	N/A	18.3
			RSV B	1.00×10^5	3/3	30.0	N/A	19.9
				2.00×10^5	3/3	31.0	N/A	18.7
Influenza B/Massachusetts/02/2012	20	29.3	Flu A	2.00×10^4	0/3	18.9	Neg	N/A
				1.00×10^4	0/3	20.1	Neg	N/A
				2.00×10^3	3/3	22.6	29.2	N/A
			RSV A	1.00×10^5	3/3	N/A	29.7	18.0
			RSV B	2.00×10^5	3/3	N/A	31.2	18.6
				1.00×10^5	3/3	N/A	30.7	18.6
RSV A2	40	29.8	Flu A	2.00×10^4	0/3	19.1	N/A	Neg
				1.00×10^4	2/3	20.4	N/A	34.2
				2.00×10^3	3/3	22.3	N/A	31.5
			Flu B	5.00×10^4	3/3	N/A	15.2	34.1
RSV B	80	28.8	Flu A	2.00×10^4	0/3	19.4	N/A	Neg
				1.00×10^4	0/3	19.9	N/A	Neg
				2.00×10^3	3/3	22.6	N/A	30.8
			Flu B	5.00×10^4	3/3	N/A	15.2	30.3
				2.50×10^4	3/3	N/A	16.1	29.4
				5.00×10^3	3/3	N/A	18.7	28.3

The results indicate that the assay is subject to competitive inhibition in cases of co-infection, where high levels of influenza A (i.e., $>2.0 \times 10^3$ $TCID_{50}$/mL) will likely suppress the detection of influenza B or RSV when those viruses are present at low levels. A limitation to the labeling has been to disclose this vulnerability of the assay to competitive inhibition from Flu A.

Interfering Substances

The performance of the Simplexa™ Flu A/B & RSV Direct Gen II assay was evaluated with potentially interfering substances that may be present in nasopharyngeal passages. A total of 10 potentially interfering substances were individually spiked into a pooled nasopharyngeal swab matrix containing influenza A (Influenza A/Hong Kong/8/68), influenza B (Influenza B/Massachusetts/02/2012) and RSV (A2), each at a targeted concentration approximately 2x LoD; each sample was tested in three replicates. No interference was observed with the substances at the concentrations shown below.

Interference of Endogenous and Exogenous Substances on Simplexa Flu A/B & RSV Direct Gen II

Potential Interferent	Active Ingredient	Interferent Concentration	% Detection		
			Flu A	Flu B	RSV
Afrin Nasal Spray	Oxymetazoline	15% (v/v)	100%	100%	100%
Antibacterial, systemic	Tobramycin	4 µg/mL	100%	100%	100%
Antibiotic, nasal ointment	Mupirocin	3.3 mg/mL	100%	100%	100%
Blood	N/A	2% (v/v)	100%	100%	100%
Bovine Submaxillary Gland Type I-S	Purified Mucin Protein	30 µg/mL	100%	100%	100%
Nasal Corticosteroid - Beconase AQ	Beclomethasone	5% (v/v)	100%	100%	100%
Nasal Corticosteroid - Fluticasone	Fluticasone	5% (v/v)	100%	100%	100%
Relenza Antiviral Drug	Zanamivir	3.3 mg/mL	100%	100%	100%
Tamiflu Antiviral Drug	Oseltamivir	1 µM	100%	100%	100%
Zicam Nasal Gel	Luffa Opperculata, Galphimia glauca, histaminum hydrochloricum	5% (v/v)	100%	100%	100%

5. Specimen Stability

Specimens Collected in UTM

Specimen stability was evaluated when samples were collected in UTM. Test samples were prepared at the concentrations relative to the LoD for each virus, ranging from approximately 2x LoD to 50x LoD, as shown below.

Specimen Stability Test Panel

Sample	Flu A $TCID_{50}$/mL (copies/mL)	Flu B $TCID_{50}$/mL (copies/mL)	RSV $TCID_{50}$/mL (copies/mL)	No. of Reps. (per time point)
2x LoD	4.3 (5,162)	10.6 (2,029)	21.3 (1,974)	46
4x LoD	8.0 (9,716)	20.0 (3,820)	40.0 (3,716)	5
10x LoD	20.0 (24,290	50.0 (9,550)	100.0 (9,290)	5
20x LoD	40.0 (48,580)	100.0 (19,100)	200.0 (18,580)	5
50x LoD	100.0 (12,1450)	250.0 (47,750)	500.0 (46,450)	5

The samples were aliquoted and stored at 2-8°C for 3 days and 7 days. After 7 days of storage in refrigerator, the samples were transferred to a freezer at ≤-70°C. Frozen samples were subjected to four freeze/thaw cycles and then were tested with the Simplexa Flu A/B & RSV Direct Gen II assay. The study utilized five LIAISON MDX instruments and one lot of

the Reaction Mix. At each study time-point, the test samples were removed from the storage conditions and equilibrated to room temperature prior to testing. The stability was evaluated by the change in Ct values from baseline Ct (at day 0).

For Flu A in UTM samples:

a. After storage at 2-8°C for 7 days, the observed average change from the baseline Ct, across all concentration levels, ranged from -0.1 to 0.1 Ct.
b. After 4 F/T cycles (7-day storage at 2-8°C followed by storage at -70°C), the observed average change from the baseline Ct, across all concentration levels, ranged from 0.1 to 0.6 Ct.

For Flu B in UTM samples:

a. after storage at 2-8°C for 7 days, the observed average change from the baseline Ct, across all concentration levels, ranged from -0.1 to 0.5 Ct.
b. After 4 F/T cycles (7-day storage at 2-8°C followed by storage at -70°C), the observed change from the baseline Ct, across all concentration levels, ranged from 0.0 to 0.3 Ct.

For RSV in UTM samples:

a. after storage at 2-8°C for 7 days, the observed average change from the baseline Ct, across all concentration levels, ranged from -0.1 to 0.1 Ct.
b. After 4 F/T cycles (7-day storage at 2-8°C followed by storage at -70°C), the observed average change from the baseline Ct, across all concentration levels, ranged from -0.3 to 0.1 Ct.

The results demonstrated acceptable stability of samples collected in UTM when stored at the above conditions for the time period under evaluation.

Specimens Collected in Other Collection Media

Additionally, specimen stability was evaluated when samples were collected in three other collection media: Copan ESwab Liquid Amies, Remel M5, and Remel M6. The test samples were prepared by diluting viral stocks of Flu A, Flu B, and RSV into each of the selected media at concentrations ranging from approximately 2x LoD to 50x LoD. The samples were aliquoted and stored at 2-8°C for three days, then frozen at ≤-70°C, to evaluate three freeze/thaw cycles. The study utilized six LIAISON MDX instruments and one lot of the Reaction Mix. At each study time-point, the test samples were removed from the storage conditions and equilibrated to room temperature prior to testing.

Summary of results from the stability evaluation of samples collected in three other media, is shown below.

Summary of Sample Stability (other Media)

		Change in Average Ct Values, from the Baseline					
		Flu A		Flu B		RSV	
	Conc. Level	After 3 Days at 2-8°C	After 3 F/T Cycles (at -70°C)	After 3 Days at 2-8°C	After 3 F/T Cycles (at -70°C)	After 3 Days at 2-8°C	After 3 F/T Cycles (at -70°C)
Liquid Amies	2x LoD	0.0	0.4	-0.7	0.7 to 1.7	1.0	0.7
	4x LoD	1.0	1.1	-0.2	0.7	1.1	2.4
	>4x LoD	0.3 to 0.8	0.6 to1.4	1.3 to 1.7	1.3 to 1.7	0.9 to 1.5	1.2 to 1.6
Remel 5	2x LoD	0.1	0.6	-0.4	-0.1	0.0	0.1
	4x LoD	0.0	0.5	0.0	0.1	-0.1	0.5
	>4x LoD	0.5 to 0.7	0.4 to 0.8	0.0 to 0.2	0.0 to 0.2	-0.1 to 0.5	-0.4 to 0.3
Remel 6	2x LoD	0.2	0.4	0.3	0.4	0.1	0.4
	4x LoD	0.9	1.2	-0.3	-0.2	0.1	0.6
	>4x LoD	0.3-0.4	0.3 to 0.5	-0.1 to 0.0	-0.2 to 0.2	-0.3 to 0.2	-0.1 to 0.9

The results demonstrated acceptable stability of samples collected in the three types of media tested, when stored at the above conditions for the time period under evaluation.

The package insert includes the following storage and transport recommendations:

- Specimens collected in UTM should be transported on ice and may be stored at 2 to 8°C for up to seven (7) days.
- Specimens collected in Remel M5, Remel M6, or Copan ESwab™ (Liquid Amies) should be transported on ice and may be stored at 2 to 8°C for up to three (3) days.

6. Reaction Mix Open Vial Stability at Room Temperature

The stability of the Reaction Mix after opening was evaluated for room temperature stability at 30 and 60 minutes timepoints. The study was conducted using Positive Control neat and diluted 1:10 in UTM, testing two lots of reagents across two LIAISON MDX instruments. Each neat sample was tested in duplicate at each time point with each lot of reagents. The 1:10 diluted sample was tested in 12 replicates at each timepoint with each lot of reagents. All replicates were detected at all timepoints (0 (after preparation), 30 minutes and 60 minutes) and the observed change in Ct values was ≤ 1.2% for all three targets.

7. Quality Controls

External controls, the Simplexa™ Flu A/B & RSV Positive Control Pack, are available from DiaSorin and may be used to monitor the assay performance and integrity of the reagents over time.

Three lots of Positive Control were included in the evaluation of the Simplexa Flu A/B & RSV Direct Gen II assay. The summary of the QC results is presented below.

Summary of the QC Data Overall

Target Analyte	Total N	Avg. Ct	SD	%CV
Flu A	169	25.5	0.6	2.33
Flu B	169	23.7	0.5	1.94
RSV	169	25.9	0.6	2.29
IC	169	28.7	1.0	3.52

8. Detection Limit:

The Limit of Detection (LoD) study was performed using six LIAISON MDX Instruments, two lots of Reaction Mix and two lots of Positive Control. A total of 67 runs were performed by two operators over three days. The test samples were prepared by diluting viral stocks of influenza A, influenza B, and RSV into pooled nasopharyngeal swab (NPS) specimens collected in UTM. Two strains of each virus were tested. The viral stocks were previously titered in $TCID_{50}$/mL (Tissue Culture Infectious Dose at 50% per mL) and in copies/mL using digital droplet PCR technique. Each of the virus strains was tested at four or more concentrations near the expected LoD during the initial screening, followed by confirmation testing at one or more concentrations with two lots of the Reaction Mix, in 20 replicates. The LoD was defined as the concentration of the virus that resulted in at least 95% detection during confirmation testing for each of the virus strains. The confirmed LoD values are shown below.

Claimed LoD for Simplexa Flu A/B &RSV Direct Gen II

Virus	$TCID_{50}$/mL	Copies/mL	No. Detected	Avg. Ct
Influenza A/PR/8/34	0.05	825	19/20	33.7
Influenza A/Hong Kong/8/68	2	2,429	20/20	32.8
Influenza B/Malaysia/2506/2004	0.1	409	20/20	32.4
Influenza B/Massachusetts/02/2012	5	955	20/20	32.0
RSV-A2	10	929	20/20	31.9
RSV-B CH93-18(18)	20	6,492	20/20	30.5

9. Analytical Reactivity (Inclusivity)

The analytical reactivity of the Simplexa Flu A/B & RSV Direct Gen II with various strains of influenza A, influenza B and RSV, was evaluated by testing dilutions of quantified virus stocks prepared by spiking into into pooled negative nasopharyngeal swab matrix (swabs collected in UTM) at concentrations near the LoD. A total of 65 Flu A strains, 25 Flu B strains and 11 RSV strains were tested in three replicates. Samples that were not detected in all three replicates at the initially contrived concentrations were retested at a higher concentration. The strains shown below were positive in all three replicates at the concentrations shown.

Simplexa Flu A/B & RSV Direct Gen II Analytical Reactivity with Flu A Strains

Subtype	Organism	Tested Concentration[1]
(H1N1) pdm09	A/California/4/2009	100 $TCID_{50}$/mL
(H1N1) pdm09	A/California/7/2009	100 $CEID_{50}$/mL
(H1N1) pdm09	A/California/12/2012	100 $TCID_{50}$/mL
(H1N1) pdm09	A/Massachusetts/15/2013	1000 $CEID_{50}$/mL
(H1N1) pdm09	A/Mexico/4108/2009	100 $CEID_{50}$/mL
(H1N1) pdm09	A/New York/18/2009	100 $CEID_{50}$/mL
H1N1	A/Brisbane/59/07	100 $TCID_{50}$/mL
H1N1	A/Hawaii/15/2001	100 $CEID_{50}$/mL
H1N1	A/New Caledonia/20/99	100 $TCID_{50}$/mL
H1N1	A/Solomon Island/3/2006	100 $TCID_{50}$/mL
H1N1	A/Taiwan/42/06	100 $TCID_{50}$/mL
H1N1	A/WS/33	100 $TCID_{50}$/mL
H2N2	A/Japan/305/57	0.326 ng/µL[2]
H3N2	A/Brisbane/10/07	100 $TCID_{50}$/mL
H3N2	A/California/02/2014	100 $CEID_{50}$/mL
H3N2	A/New York/55/2004	100 $CEID_{50}$/mL
H3N2	A/Ohio/02/2012	200 $CEID_{50}$/mL
H3N2	A/Port Chalmers/1/1973	100 $TCID_{50}$/mL
H3N2	A/Rhode Island/01/2010	400 $CEID_{50}$/mL
H3N2	A/Santiago/7981/2006	100 $CEID_{50}$/mL
H3N2	A/Switzerland/9715293/2013	200 $CEID_{50}$/mL
H3N2	A/Texas/50/2012	100 $CEID_{50}$/mL
H3N2	A/Wisconsin/67/05	100 $TCID_{50}$/mL
H3N2v	A/Indiana/08/2011	100 $CEID_{50}$/mL
H3N2v	A/Minnesota/11/2010	100 $CEID_{50}$/mL
H5N1	A/Egypt/N03072/2010(H5N1)-PR8-IDCDC-RG29	1:100,000 dilution
H5N1	A/Hubei/1/2010(H5N1)-PR8-IDCDC-RG30	1:100,000 dilution
H5N1	A/India/NIV/2006(H5N1)-PR8-IBCDC-RG7	1:100,000 dilution
H7N9	A/Anhui/1/2013	1:100,000 dilution

Subtype	Organism	Tested Concentration[1]
H9N2	A/Hong Kong/33982/2009(H9N2)-PR8-IDCDC_RG26	100 $CEID_{50}$/mL
H1N3	A/shorebird/Delaware Bay/211/1994	100 $CEID_{50}$/mL
H1N8	A/red knot/Delaware Bay/240/1994	200 $CEID_{50}$/mL
H3N6	A/redhead/Alberta/192/2002	100 $CEID_{50}$/mL
H3N8	A/duck/Chabarovsk/1610/1972	400 $CEID_{50}$/mL
H4N6	A/duck/Czechoslovakia/1956	500 $CEID_{50}$/mL
H4N6	A/red knot/Delaware/541/1988	200 $CEID_{50}$/mL
H5N1	A/chicken/Vietnam/NCVD-016/2008(H5N1)-PR8-IDCDC-RG12	1:100,000 dilution
H5N2	A/pheasant/New Jersey/1355/1998(H5N2)-PR8-IBCDC-4	1:100,000 dilution
H6N2	A/turkey/Massachusetts/3740/1965	800 $CEID_{50}$/mL
H7N2	A/turkey/Virginia/4529/2002 (H7N2)xPR8-IBCDC-5	1:100,000 dilution
H7N7	A/mallard/Netherlands/12/2000(H7N7)/PR8-IBCDC-1	1:100,000 dilution
H10N7	A/mallard/Illinois/10OS4334/2010	100 $CEID_{50}$/mL
H10N7	A/chicken/Germany/N/49	100 $CEID_{50}$/mL
H10N1	A/mallard/Wisconsin/4230/2009	100 $CEID_{50}$/mL
H10N8	A/quail/Italy/1117/1965	100 $CEID_{50}$/mL
H11N9	A/American green-winged teal/Mississippi/300/2010	100 $CEID_{50}$/mL
H12N5	A/mallard/Wisconsin/4218/2009	100 $CEID_{50}$/mL
H12N6	A/duck/Wisconsin/480/1979	100 $CEID_{50}$/mL
H13N6	A/black-legged kittiwake/Quebec/02838-1/2009	200 $CEID_{50}$/mL
H16N3	A/shorebird/Delaware/172/2006	400 $CEID_{50}$/mL
H1N1	A/Swine/1976/31	100 $TCID_{50}$/mL
H1N1	A/Swine/Iowa/15/30	100 $TCID_{50}$/mL
H1N2	A/swine/Ohio/09SW1477/2009	100 $TCID_{50}$/mL
H3N2	A/swine/Ohio/09SW83E/2009	400 $CEID_{50}$/mL
H3N2	A/Singapore/INFIMH-16-0019/2016	100 $CEID_{50}$/mL
(H1N1) pdm09	A/Michigan/45/2015	100 $CEID_{50}$/mL
H1N2	A/Minnesota/19/2011	1000 $CEID_{50}$/mL

Subtype	Organism	Tested Concentration[1]
H3N2	A/Hong Kong/4801/2014	200 $CEID_{50}$/mL
H3N2	A/Kansas/14/2017	100 EID_{50}/mL
(H1N1) pdm09	A/Brisbane/02/2018	100 EID_{50}/mL
(H1N1) pdm09	A/Christ Church/16/2010	200 EID_{50}/mL
H3N2	A/Hong Kong/2671/2019	50 EID_{50}/mL
(H1N1) pdm09	A/Guangdong-Maonan/1536/2019	100 EID_{50}/mL
H3N2	A/Perth/16/2009	50 EID_{50}/mL
(H1N1) pdm09	A/NY/02/09	0.04 $TCID_{50}$/mL

[1] $TCID_{50}$/mL = Tissue Culture Infectious Dose
$CEID_{50}$/mL = Chicken Embryo Infectious Dose
EID_{50}/mL = Egg Infectious Dose

[2] Flu A/Japan/305/57 was in the form of unprotected genomic RNA stock and was prepared in Tris-EDTA (TE) buffer.

Simplexa™ Flu A/B & RSV Direct Gen II Analytical Reactivity with Flu B Strains

Lineage	Organism	Tested Concentration[1]
Victoria	B/Brisbane/33/2008	20 $CEID_{50}$/mL
Victoria	B/Brisbane/60/2008	20 $CEID_{50}$/mL
Victoria	B/Florida/02/2006	100 $TCID_{50}$/mL
Victoria	B/Lee/40	100 $TCID_{50}$/mL
Victoria	B/Nevada/03/2011	100 $CEID_{50}$/mL
Victoria	B/Texas/02/2013	100 $TCID_{50}$/mL
Victoria	B/Victoria/304/2006	50 $CEID_{50}$/mL
Yamagata	B/Christchurch/33/2004	100 $CEID_{50}$/mL
Yamagata	B/Florida/07/04	100 $TCID_{50}$/mL
Yamagata	B/Florida/04/2006	100 $TCID_{50}$/mL
Yamagata	B/Guangdong-Liwan/1133/2014	400 $CEID_{50}$/mL
Yamagata	B/Maryland/1/59	100 $TCID_{50}$/mL
Unknown	B/Great Lakes/1739/54	100 $TCID_{50}$/mL
Yamagata	B/Panama/45/90	100 $TCID_{50}$/mL
Yamagata	B/Phuket/3073/2013	200 $CEID_{50}$/mL
Yamagata	B/Utah/09/2014	100 $CEID_{50}$/mL

Lineage	Organism	Tested Concentration[1]
Yamagata	B/Wisconsin/01/2010	100 $CEID_{50}$/mL
Unknown	B/Allen/45	100 $TCID_{50}$/mL
Unknown	B/Hong Kong/5/72	100 $TCID_{50}$/mL
Unknown	B/Taiwan/2/62	100 $TCID_{50}$/mL
Victoria	B/Colorado/06/2017	20 $TCID_{50}$/mL
Victoria	B/Michigan/09/2011	5 EID_{50}/mL
Yamagata	B/Texas/81/2016	20 EID_{50}/mL
Victoria	B/Washington/02/2019	200 EID_{50}/mL
Yamagata	B/New Hampshire/01/2016	100 EID_{50}/mL

[1] $TCID_{50}$/mL = Tissue Culture Infectious Dose
$CEID_{50}$/mL = Chicken Embryo Infectious Dose
EID_{50}/mL = Egg Infectious Dose

Simplexa Flu A/B & RSV Direct Gen II Analytical Reactivity with RSV Strains

Organism	Tested Concentration[1]
RSV ATCC-2012-10	100 PFU/mL
RSV A 1997/12-35	100 $TCID_{50}$/mL
RSV A 1998/12-21	100 $TCID_{50}$/mL
RSV A 1998/3-2	100 $TCID_{50}$/mL
RSV A 2000/3-4	100 $TCID_{50}$/mL
RSV A 2001/2-20	100 $TCID_{50}$/mL
RSV A 2001/3-12	100 $TCID_{50}$/mL
RSV A Long	100 $TCID_{50}$/mL
RSV B 9320	100 $TCID_{50}$/mL
RSV B/Wash/18537/62	100 $TCID_{50}$/mL
RSV B/WV/14617/85	100 $TCID_{50}$/mL

[1] $TCID_{50}$/mL = Tissue Culture Infectious Dose
PFU/mL = Plaque Forming Units

Additionally, testing of eight contemporary human influenza viruses was conducted to compare the reactivity of the modified device (Simplexa Flu A/B & RSV Direct Gen II) to the unmodified version (Simplexa Flu A/B & RSV Direct). The viruses, obtained from CDC, were diluted in pooled negative matrix and tested in three replicates, to determine the lowest concentration detectable by each version of the assay. The results are presented below.

Type	Subtype/Lineage	Strain	Lowest Concentration detectable in 3/3 Replicates	
			Simplexa Flu A/B & RSV Direct Gen II (modified)[1]	Simplexa Flu A/B & RSV Direct (original)
A	(H1N1) pdm09	A/Christ Church/16/2010	200 EID_{50}/mL	2,000 EID_{50}/mL
A	H3N2	A/Hong Kong/2671/2019	50 EID_{50}/mL	50 EID_{50}/mL
A	(H1N1) pdm09	A/Guangdong-Maonan/1536/2019	100 EID_{50}/mL	400 EID_{50}/mL
A	H3N2	A/Perth/16/2009	50 EID_{50}/mL	50 EID_{50}/mL
B	Yamagata	B/Phuket/3073/2013[b]	200 $CEID_{50}$/mL	1,000 $CEID_{50}$/mL
B	Victoria	B/Michigan/09/2011	5 EID_{50}/mL	1,000 EID_{50}/mL
B	Yamagata	B/Texas/81/2016	20 EID_{50}/mL	100 EID_{50}/mL
B	Victoria	B/Washington/02/2019	200 EID_{50}/mL	4,000 EID_{50}/mL

[1] EID_{50}/mL = Egg Infectious Dose at 50% per mL
$CEID_{50}$/mL = Chicken Embryo Infectious Dose

Overall, the modified device demonstrated higher analytical reactivity with contemporary strains of human influenza viruses than the original device.

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10. Assay Cut-Off:

The fluorescent thresholds and assay Ct cutoffs were adjusted to optimize the sensitivity and specificity of the Simplexa Flu A/B & RSV Direct Gen II based on the LoD data and results from testing of 880 No-Template-Control samples, using three lots of the Reaction Mix reagents, across 11 LIAISON MDX instruments. The overall positive and negative percent agreements obtained from the method comparison testing with clinical samples were determined to be satisfactory.

11. Carry-Over:

The potential for carry-over contamination using the LIAISON MDX instrument was previously evaluated and reviewed under K120413. The study was performed by testing high positive and negative samples in alternate positions on each disc. No carry-over contamination effect was seen in the Flu A, Flu B or RSV channels.

An additional study was conducted with the modified device to evaluate the risk for cross-contamination and to demonstrate the re-usability of the DAD consumable up to eight times.

In the first part of the study, two lots of DADs were evaluated, testing 10 DADs/lot and utilizing 12 LIAISON MDX instruments. Each DAD was used to test two wedges at a time

(alternating Positive Control and No Template Control) per run, for four runs per DAD, for a total of 80 measurements for each sample.

No carryover contamination was observed. The summary results are presented below.

Carryover Contamination Summary Results

Sample	Flu A (FAM)		Flu B (JOE)		RSV (CFR610)		IC (Q670)	
	% Detection	**Mean Ct**	**% Detection**	**Mean Ct**	**% Detection**	**Mean Ct**	**% Detection**	**Mean Ct**
PC	100% (80/80)	25.1	100% (80/80)	23.7	100% (80/80)	26.0	98.8% (79/80)*	29.0
NTC	0% (0/80)	N/A	0% (0/80)	N/A	0% (0/80)	N/A	0% (0/80)	28.8

*One PC replicate did not detect IC. IC detection is not required if Flu A, Flu B or RSV is detected

In the second part of the study, four DADs were tested using Positive Control (PC) and Universal Transport Media (UTM) as a No Template Control (NTC). Since each Disc can accommodate up to eight samples, the purpose of the study was to demonstrate that each disc can be used up to eight times, without a risk of contamination Each wedge of the DAD was run one wedge at a time per run for a total of eight runs per DAD and 16 replicates of each sample.

No carryover contamination was observed. The summary results are presented below.

DAD Re-usability Study Results

Sample	Flu A (FAM)		Flu B (JOE)		RSV (CFR610)		IC (Q670)	
	% Detection	Mean Ct	% Detection	Mean Ct	% Detection	Mean Ct	% Detection	Mean Ct
PC	100% (16/16)	25.2	100% (16/16)	23.6	100% (16/16)	25.5	100% (16/16)	28.2
NTC	0% (0/16)	N/A	0% (0/16)	N/A	0% (0/16)	N/A	100% (16/16)	27.8

B Comparison Studies:

1. Method Comparison with Predicate Device:

A clinical agreement study was performed using retrospective pre-selected positive and negative leftover nasopharyngeal swab (NPS) specimens collected in 3 mL Universal Transport Media (UTM) from patients with signs and symptoms of respiratory tract infection. The specimens were collected at three collection sites at two geographical locations. A total of 240 pre-selected positive and negative nasopharyngeal swabs, collected between January 24, 2018 and December 15, 2019, were included in the evaluation. The specimens were shipped on dry ice and stored at ≤ -70°C until testing. All specimens were pre-selected for positive and negative status based on routine laboratory results at the collection sites. The specimens were re-blinded and randomized prior to distributing them to the internal clinical testing site at DiaSorin Molecular. Each specimen was tested using the predicate device, Simplexa Flu A/B & RSV Direct, and the modified device, Simplexa Flu

A/B & RSV Direct Gen II. Any sample with discordant results was tested with another FDA cleared molecular assay. Six LIAISON MDX instruments were used in the evaluation.

The following tables show the results from the method comparison testing.

Influenza A

Simplexa Flu A/B & RSV Direct Gen II (modified)	**Simplexa Flu A/B & RSV Direct (predicate)**		**Total**
	Detected	**Not Detected**	
Detected	55	4[a]	59
Not Detected	0	181	181
Total	55	185	240
PPA = 100.0% (55/55) 95% CI: 93.5% to 100%	NPA = 97.8% (181/185) 95% CI: 94.6% to 99.2%		

[a] All four samples negative by the predicate and positive by the modified assay, were positive by another FDA cleared NAAT.

Influenza B

Simplexa Flu A/B & RSV Direct Gen II (modified)	**Simplexa Flu A/B & RSV Direct (predicate)**		**Total**
	Detected	**Not Detected**	
Detected	58	4[a]	62
Not Detected	0	178	178
Total	58	182	240
PPA = 100.0% (58/58) 95% CI: 93.8% to 100%	NPA = 97.8% (178/182) 95% CI: 94.5% to 99.1%		

[a] Of the four samples negative by the predicate and positive by the modified assay, one was positive by another FDA cleared NAAT.

RSV

Simplexa Flu A/B & RSV Direct Gen II (modified)	Simplexa Flu A/B & RSV Direct (predicate)		Total
	Detected	Not Detected	
Detected	52	6[a]	58
Not Detected	0	182	182
Total	52	188	240
PPA = 100.0% (52/52) 95% CI: 93.1% to 100%	NPA = 96.8% (182/188) 95% CI: 93.2% to 98.5%		

[a]All six samples negative by the predicate and positive by the modified assay, were positive by another FDA cleared NAAT.

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

The clinical performance of the Simplexa Flu A/B & RSV Direct (unmodified device) was established in comparison to viral culture, during a clinical study conducted between September 2010 and March 2011 (see K120413).

Clinical Performance of the Simplexa Flu A/B & RSV Direct Prospective Data (K120413)

	Sensitivity	95% CI	Specificity	95% CI
Flu A	97.1% (66/68)	89.9% to 99.2%	97.9% (639/653)	96.4% to 98.7%
Flu B	100.0% (21/21)	84.5% to 100.0%	99.9% (697/698)	99.2% to 100.0%
RSV	97.6% (82/84)	91.7% to 99.4%	94.4%* (592/627)	92.3% to 96.0%

*Of the 45 samples positive by the Simplexa Flu A/B & RSV Direct and negative by culture for RSV, 35 samples were positive for RSV by another molecular assay.

2. Other Clinical Supportive Data:

See Comparison Studies, section B above.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

The original Simplexa Flu A/B & RSV Direct was evaluated during the 2010/2011 influenza season and that data was previously presented in the Decision Summary for K120413. The same data is shown below, for completeness of the record.

The positivity rates observed for influenza A, influenza B and RSV during the clinical study conducted between September2010 and March 2011are shown below. The observed prevalence (as determined by reference method) is included.

Positivity Rates for Simplexa Flu A/B & RSV Direct (2010-2011) (K120413)

Australia (n=330)					
Analyte	**Total (Prevalence)**	**< 5 years (n=63)**	**5-21 years (n=106)**	**22-60 years (n=147)**	**> 60 years (n=14)**
Influenza A	2.7% (9/330)	0	5	4	0
Influenza B	2.9% (7/330)	3	2	2	0
Respiratory Syncytial Virus	0.7% (1/330)	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)**
Influenza A	11.4% (28/245)	14	14	0	0
Influenza B	1.2% (3/245)	1	2	0	0
Respiratory Syncytial Virus	29.8% (73/245)	70	3	0	0
Virginia (n=147)					
Anayte	**Total (Prevalence)**	**< 5 years (n=69)**	**5-21 years (n=69)**	**22-60 years (n=9)**	**> 60 years (n=0)**
Influenza A	21% (31/147)	10	20	1	0
Influenza B	7.5% (11/147)	1	10	0	0
Respiratory Syncytial Virus	7.5% (11/147)	9	2	0	0

VII Proposed Labeling:

The labeling is compliant with 21 CFR 809.10 and is sufficient.

VIII Conclusion:

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