510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K163628

B. Purpose for Submission:

To establish substantial equivalence to a predicate device and to obtain clearance for a new assay: the Idylla Respiratory (IFV-RSV) Panel.

C. Measurand:

Influenza A Hemagglutinin gene (HA) RNA

Influenza A Neuraminidase gene (NA) RNA

Influenza A and Influenza B Matrix gene (M) RNA

Respiratory Syncytial Virus (RSV) fusion protein gene RNA

D. Type of Test:

Qualitative nucleic acid amplification assay for the amplification and detection of specific Influenza A, Influenza B, RSV A and RSV B RNA sequences

E. Applicant:

Janssen Diagnostics

F. Proprietary and Established Names:

Idylla Respiratory (IFV-RSV) Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3980, Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:

   Class II

3. Product code:

   OCC – Respiratory Virus Panel Nucleic Acid Assay System

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Idylla Respiratory (IFV-RSV) Panel is an in vitro assay intended for the qualitative detection of nucleic acids for Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1, H275Y mutation of Influenza A subtype 2009 H1, Influenza B and Respiratory Syncytial Virus (A and B) from nasopharyngeal swabs in viral transport media of adult and pediatric patients. The test uses the Idylla system to aid in the diagnosis of respiratory viral infection when used in conjunction with other clinical and laboratory findings.

   Negative results do not preclude respiratory virus infection or co-infection with other viruses and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

   Performance characteristics for Influenza A were established when influenza A/2009 H1 and H3 were the predominant influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL3+ 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

4. Special instrument requirements:

   To be used only with the Idylla instrument

I. Device Description:

The Idylla Respiratory Panel is a self-contained molecular *in vitro* diagnostic test intended to work with the Idylla System. The assay is performed using a single-use, disposable, multi-chambered fluidic cartridge. The assay process includes automated sample preparation/ purification, reverse transcription and real-time, multiplex Polymerase Chain Reaction (PCR) for the detection of viral RNA. All steps in this process are all fully automated and completely integrated; results are available in less than 50 minutes.

To perform the assay, a nasopharyngeal (NPS) sample is added to the cartridge lysis pad. The cartridge lid is then closed and placed in the cartridge tray on the Idylla Instrument. Once the tray is closed, the test begins automatically. Once started, cartridge manifolds move the specimen and reagents systematically through the cartridge fluidics from sample lysis, nucleic acid purification, mixing of PCR reagents, and finally amplification and signal detection. All amplification, detection of fluorescence, and the interpretation of the signals are done automatically by the Idylla System. The Idylla Console provides a qualitative result for the presence (Detected) or absence (Not Detected) for influenza A, influenza B, RSV, and subtyping information for influenza A positive samples, including H1, H3, 2009 H1, and H275Y. Results are displayed on the Idylla Console and uploaded to the laboratory information system.

Interpretation of results:

Each of the amplified targets has a unique type of fluorophore attached to their respective probe that is cleaved by the exonuclease activity of the Taq polymerase enzyme allowing for the identification and differentiation of the target viruses. Each gene marker is detected using fluorescent molecules with different excitation and emission wavelengths. Light generated by the released fluorophore(s) is captured by a photodetector and is translated into a fluorescent signal.

Quality Control

The Idylla Respiratory Panel test contains a sample processing control (SPC) in each cartridge. The SPC checks for adequate sample processing and downstream amplification and is co-amplified in each reaction chamber to ensure no PCR inhibitors were present in the sample. A SPC signal is required in every chamber for a "negative" result to be reported. In the event that no influenza or RSV signal is detected and there is no SPC signal, the cartridge is "invalid". In positive samples where target amplification is strong, the SPC is ignored and the target amplification serves as the 'control' to confirm that the sample was not inhibitory and that assay reagent performance was robust.

Negative and positive controls should be run according to local, state, and federal accrediting organization requirements.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Verigene RV+

2. Predicate 510(k) number(s):
   K103209

3. Comparison with predicate:

Table 1: Comparison of the IFV-RSV to the Predicate

	IFV-RSV	Verigene RV+
510(k) Number	K163628	K103209
Assay Targets	Influenza A, Influenza A subtypes H1, H3, H1 2009, H1 2009 with H275Y genotype, Influenza B, and RSV	Influenza A, Influenza A subtypes H1, H3, H1 2009, Influenza B, and RSV A and RSV B
Product Code	OCC	OCC
Specimen Type	NPS in VTM	NPS
Device Technology	RT-PCR nucleic acid amplification	RT-PCR nucleic acid amplification
Results Interpretation	Automated	Same
Instrument	Idylla Instrument	Verigene Instrument
Intended Use	The Idylla Respiratory (IFV-RSV) Panel is an in vitro assay intended for the qualitative detection of nucleic acids for Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1, H275Y mutation of Influenza A subtype 2009 H1, Influenza B and Respiratory Syncytial Virus (A and B) from nasopharyngeal swabs in viral transport media of adult and pediatric patients. The test uses the Idylla system to aid in the diagnosis of respiratory viral infection when used in conjunction with other clinical and laboratory findings. Negative results do not preclude	The Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) on the Verigene System is a qualitative nucleic acid multiplex test intended to simultaneously detect and identify multiple respiratory virus nucleic acids in nasopharyngeal (NP) swab specimens from individuals with signs and symptoms of respiratory tract infection. The following virus types and subtypes are identified using the RV+: Influenza A, Influenza A subtype H1, Influenza A subtype H3, 2009 H1N1, Influenza B, Respiratory Syncytial Virus (RSV) subtype A, and RSV subtype B. The test is not intended to detect Influenza C virus. Detecting and

	respiratory virus infection or co-infection with other viruses and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Performance characteristics for Influenza A were established when influenza A/2009 H1 and H3 were the predominant influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL3+ facility is available to receive and culture specimens.	identifying specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection, if used in conjunction with other clinical and laboratory findings. Negative results for Influenza A, Influenza B, or RSV do not preclude influenza virus or RSV infection and should not be used as the sole basis for diagnosis, treatment, or patient management decisions. Conversely, positive results do not rule-out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection. Performance characteristics for Influenza A Virus were established when Influenza A/H3, A/H1, and 2009 H1N1 were the predominant Influenza A viruses circulating. These characteristics may vary when other Influenza A viruses are emerging. 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 used specifically 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.

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

Not applicable.

L. Test Principle:

RT-PCR nucleic acid amplification

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility Study

A reproducibility study of the IFV-RSV was conducted by operators from three sites using panels of coded specimens containing negative, near the cutoff (C_{20}-C_{80}), low positive (at the limit of detection), and moderate positive (above the limit of detection) panel analyte samples. Two Operators tested three replicates each twice daily for six days for a total of 36 tests per site for each panel member at a specific analyte concentration.

Participants tested multiple samples of each panel member on six different days. The percent agreement with expected results was 100% for all panel members except influenza B in the moderate positive and low positive test categories (Influenza B low positive overall agreement was 99.1%). All of the true negative samples (108) generated negative test results. There were no significant differences observed within run (replicates tested by one operator), between run (five different days), between sites (three sites), or between operators (six operators).

The Reproducibility Study site-to-site qualitative results (agreements with expected results) are presented in the table below.

Table 2: IFV-RSV - Overall Reproducibility for All Three Sites for All Samples

Target[1]	Panel Member	Site 1 Agreement[2]	Site 2 Agreement	Site 3 Agreement	Overall Agreement and (Percentage)
Influenza A H1 2009 H275Y	Moderate Positive	36/36	36/36	36/36	108/108 (100%)
	Low Positive	36/36	36/36	36/36	108/108 (100%)
	Negative	36/36	35/35	36/36	107/107 (100%)
Influenza A H1	Moderate Positive	36/36	36/36	36/36	108/108 (100%)
	Low Positive	36/36	36/36	36/36	108/108 (100%)
	Near cutoff	16/36	18/35	24/36	58/107 (54.2%)
	Negative	36/36	36/36	36/36	108/108 (100%)
Influenza A H3	Moderate Positive	36/36	36/36	36/36	108/108 (100%)
	Low Positive	36/36	36/36	36/36	108/108 (100%)
	Near cutoff	24/36	21/35	24/36	69/107 (64.5%)
	Negative	36/36	36/36	36/36	108/108 (100%)
Influenza A H1 2009	Moderate Positive	36/36	36/36	36/36	108/108 (100%)
	Low Positive	36/36	36/36	36/36	108/108 (100%)
	Near cutoff	22/36	24/35	21/36	67/107 (62.6%)
Influenza B	Moderate Positive	36/36	36/36	36/36	108/108 (100%)
	Low Positive	36/36	35/36	36/36	107/108 (99.1%)
	Near cutoff	18/36	18/35	23/36	59/107 (55.1%)
	Negative	36/36	36/36	36/36	108/108 (100%)
RSV	Moderate Positive	36/36	36/36	36/36	108/108 (100%)
	Low Positive[3]	36/36	36/36	36/36	108/108 (100%)
	Low Positive[4]	36/36	36/36	36/36	108/108 (100%)
	Near cutoff	34/36	30/35	31/36	95/107 (88%)

[1] All Influenza A subtype targets that produced a positive result also produced a positive "Flu A" result
[2] Agreement for Negative panel members expressed as correctly detecting no analyte
[3] LP panel created with RSV A
[4] LP panel created with RSV B

b. Linearity/assay reportable range:

Not applicable; this is a qualitative assay.

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

Specimen Stability

To provide data supporting the specimen storage recommendations stated in the product package insert, an analytical study was carried out to evaluate specimen stability.

Positive specimens were freshly diluted into a simulated negative background matrix spiked into VTM to approximate the required target concentrations at a moderate positive (5x LoD), and tested in replicates of 10 per condition. An additional subset of 10 negative samples was analyzed per sample per condition. A total of 60 cartridges were tested (30 positive and 30 negative). All specimens were tested with the Idylla System on the day the samples were prepared. Test conditions for this study were: 0 hours, 2 hours at room temperature (15-30°C), and 24 hours refrigerated at 2-8°C. Results of the specimen stability study are shown in the tables below.

Tables 3 and 4: Specimen Stability of the IFV-RSV Panel

Storage Condition	Agreement (Moderate Positive)	% Positive Agreement	Results			
			Flu A	Flu B	2009 H1	RSV
0 Hours	10/10	100%	+	+	+	+
2 Hours 15-30°C	10/10	100%	+	+	+	+
24 Hours 2-8°C	10/10	100%	+	+	+	+

Storage Condition	Agreement (Negative Samples)	% Negative Agreement	Results			
			Flu A	Flu B	2009 H1	RSV
0 Hours	10/10	100%	-	-	-	-
2 Hours 15-30°C	10/10	100%	-	-	-	-
24 Hours 2-8°C	10/10	100%	-	-	-	-

All samples generated expected results for each storage condition at each storage time point.

The Product Insert states that samples can be stored in VTM at room temperature for up to 2 hours or at 2-8 °C for up to 24 hours.

Fresh versus Frozen Performance Equivalency

This study was performed with contrived positive and negative specimens. A background matrix was made using negative human donor matrix samples, re-suspended in VTM for NPS testing. The positive simulated specimens were spiked into VTM media, and serially diluted to the appropriate panel concentrations. Contrived negative specimens consisted of simulated background matrix only and were added to VTM. For NP samples, contrived specimens were tested fresh (at time of serial dilutions) and after 2 freeze/thaw cycles (2 F/T) for a total of 30 paired tests for the low (2x LoD) concentration panel members. Each freeze/thaw cycle consisted of a minimum of 48 hours at ≤-70°C, followed by a thaw at 15-30°C for 1 hour prior to re-freezing or testing. Positive specimens in the study consisted of a panel of the 3 viral strains, representing the target viruses. Influenza A 2009 H1N1, Influenza B, and RSVA were selected as representative markers. Results for testing the low concentration panel members are shown in the table below.

Table 5: Fresh Versus Frozen Results for the IFV-RSV

Panel Member	Number correct/Expected (Fresh)	Number correct/Expected (2 Freeze/Thaws)
Influenza A	30/30	30/30
Influenza B	30/30	30/30
2009 H1N1	30/30	30/30
RSV	30/30	30/30

Shelf Life

Janssen performed a product stability study to generate stability data to support the expiry assigned to IFV-RSV test kits. Three lots of Idylla cartridges were tested in support of claimed shelf life. The product lots were tested minimally at Month 0 (QC Release), 6, 7, 12, and 13 months. Testing occurred within 2 weeks of the targeted date. The Idylla Respiratory (IFV-RSV) Panel is stable when unopened up to 13 months. Janssen intends to test additional time points to extend the shelf life.

Swab Type Compatibility Study

Janssen performed a swab type validation study to establish which swabs are acceptable for use with the IFV-RSV assay. Swabs tested in this study include: Puritan HydraFlock Flocked Swab, Puritan UltraFlock, Copan Flocked Swab; Copan Pediatric Mid Turbinate Flocked Swab; and Copan Adult Mid Turbinate Flocked Swab. Positive specimens in this study were freshly prepared. Positive specimens were tested in replicates of 10 per each specimen (5x LoD) and swab type (n=5); along with negative specimens as controls. A total of 100 cartridges were tested (50 positive and 50 negative). All specimens were tested with the Idylla system on the same day as preparation. Agreement at 100% was demonstrated for both 5x LoD and negative specimens when tested using all Copan and Puritan flocked swabs tested.

Viral Transport Media Validation Study

Janssen performed a study to determine the validity of commonly used viral transport media for use with the Idylla Respiratory Panel. Contrived positive specimens consisted of cultured viral targets diluted into a simulated background matrix (negative matrix). This background matrix was made using pooled negative human donor swab samples re-suspended in one of the VTMs. Each pool of negative matrix was tested to confirm the absence of influenza and RSV prior to the study. All replicates were correctly called negative in each type of media used. The positive specimens were diluted into the negative background matrix at a 1000x LoD concentration, then diluted in the appropriate medium under investigation to approximate the required target concentrations at a moderate positive (5x LoD). Each panel simultaneously detected FluA, 2009H1, FluB, and RSVA. The positive specimens were diluted into the negative background matrix made for each media type and tested in replicates of 10 per each specimen (5x LoD and Negative) and media (n=6). A total of 120 cartridges were tested (60 positive and 60 negative). All specimens were tested with the Idylla System on the day they were prepared. Agreement at 100% was demonstrated for both 5x LoD and negative specimens when tested using Remel media (M4, M4-RT, M5, M6), Copan UTM, and Cepheid GeneXpert Influenza Virus VTM. Results are shown in the tables below.

Tables 6 and 7: VTM Validation Study Results for the IFV-RSV Panel

Media	Agreement (Moderate Positive)	% Positive Agreement	Results			
			Flu A	Flu B	2009 H1	RSV
Remel M4	10/10	100%	+	+	+	+
Remel M4-RT	10/10	100%	+	+	+	+
Remel M5	10/10	100%	+	+	+	+
Remel M6	10/10	100%	+	+	+	+
Copan UTM	10/10	100%	+	+	+	+
Cepheid GeneXpert VTM	10/10	100%	+	+	+	+

Media	Agreement (Negative Samples)	% Negative Agreement	Results			
			Flu A	Flu B	2009 H1	RSV
Remel M4	10/10	100%	-	-	-	-
Remel M4-RT	10/10	100%	-	-	-	-
Remel M5	10/10	100%	-	-	-	-
Remel M6	10/10	100%	-	-	-	-
Copan UTM	10/10	100%	-	-	-	-
Cepheid GeneXpert VTM	10/10	100%	-	-	-	-

d. Detection limit:

The objective of the Analytical Sensitivity Study was to identify the limit of detection of the IFV-RSV using characterized strains of each analyte target. The analytical sensitivity was determined using quantified cultures of 8 Influenza A (2 each of AH1, AH3, 2009 H1 and 2009 H1 + H275Y Genotype), 2 Influenza B, 2 Respiratory Syncytial Virus Type A (RSVA), and 2 Respiratory Syncytial Virus Type B (RSVB) strains serially diluted. The LoD for each strain was defined as the lowest concentration at which ≥95% of the 20 tested replicates were detected by the system for each clinical sample type.

Three replicates of each dilution level were tested at 200 µL sample addition. At the lowest dilution level in which 3/3 replicates were detected, confirmation of LoD for each target was achieved by testing 20 replicates at this level with minimum reported "Detected" result in 95% of the cartridges tested (≥19/20) using 200 µL of sample. For 500 µL sample addition, 3 replicates were analyzed at the equivalent concentration as determined for LoD with 200 µL sample addition, followed by a minimum of 2 additional dilutions lower in replicates of 3. If testing of 2-fold dilutions did not generate a 95% positive result, dilutions that were half-way between the lowest 100% positive 2-fold dilution and the 2-fold dilution below it were tested until a dilution testing 95% positive was determined. The empirically determined LoD for each Influenza and RSV strain is shown in the table below.

Table 8: IFV-RSV Limits of Detection

Assay Call	Virus Strain	Limit of Detection (200 µL VTM sample) $TCID_{50}$/mL	Limit of Detection (500 µL VTM sample) $TCID_{50}$/mL
Influenza A Detected	Influenza A/New Caledonia/20/99 (H1N1)	2.5×10^{0}	1.2×10^{0}
	Influenza A/Taiwan/42/06 (H1N1)	1.7×10^{0}	6.8×10^{-1}
Influenza A Detected, Subtype Detected	Influenza A/New Caledonia/20/99 (H1N1)	3.0×10^{0}	1.2×10^{0}
	Influenza A/Taiwan/42/06 (H1N1)	1.7×10^{0}	6.8×10^{-1}
	Influenza A/Texas/50/12 (H3N2)	1.4×10^{0}	5.6×10^{-1}
	Influenza A/Victoria/361/11(H3N2)	1.4×10^{0}	5.6×10^{-1}
	Influenza A/NY/01/09 (2009 H1)	1.0×10^{0}	4.2×10^{-1}
	Influenza A/NY/03/09 (2009 H1)	7.0×10^{-1}	2.8×10^{-1}
	Influenza A/Texas/48/2009 (2009 H1 + H275Y)	9.3×10^{0}	1.0×10^{0}
	Influenza A /North Carolina/39/2009 (2009 H1N1 + H275Y)	9.3×10^{0}	1.0×10^{0}
Influenza B Detected	Influenza B/Florida/04/06	1.4×10^{-1}	5.6×10^{-2}
	Influenza B/Malaysia/2506/04	6.0×10^{-2}	2.3×10^{-2}

RSV Detected	RSVA A2	2.8×10^{0}	1.13×10^{0}
	RSVA 2006 Clinical Isolate	5.0×10^{0}	2.0×10^{0}
	RSVB Wash/18537/62	9.0×10^{-2}	4.0×10^{-2}
	RSVB CH93(18)-18	4.2×10^{-1}	1.7×10^{-1}

e. Analytical reactivity:

An analytical reactivity study was completed to determine whether the Idylla Respiratory (IFV-RSV) Panel can detect a variety of Influenza A, Influenza B, RSVA and RSVB strains that represent both temporal and geographic diversity.

The analytical reactivity (inclusivity) of the Idylla Respiratory (IFV-RSV) Panel was determined using quantified cultures of Influenza A (subtypes H1, H3, H1N12009 +/- H275Y mutation), Influenza B, RSVA and RSVB strains. The virus was diluted into a VTM containing a donor swab. Each strain was run in triplicate at 2X LoD using both sample volumes, 200 µL and 500 µL. Concentrations used for analytical reactivity (inclusivity) claims were calculated based on the reported LoD. A summary of the results of the reactivity testing is presented in the table below.

Table 9: IFV-RSV Analytical Reactivity Results

Strain	Sample Type/ Volume								
	200 µL VTM $TCID_{50}$/mL	500 µL VTM $TCID_{50}$/mL	FLUA	AH1	AH3	FLU B	2009 H1	H275Y	RSV
A/Canada/6294/09	2×10^{0}	8.4×10^{-1}	+	-	-	-	+	-	-
A/NY/02/09	2×10^{0}	8.4×10^{-1}	+	-	-	-	+	-	-
A/NY/03/09	2×10^{0}	8.4×10^{-1}	+	-	-	-	+	-	-
A/NY/01/09	2×10^{0}	8.4×10^{-1}	+	-	-	-	+	-	-
A/California/07/09	2×10^{0}	8.4×10^{-1}	+	-	-	-	+	-	-
A/Mexico/4108/09	2×10^{0}	8.4×10^{-1}	+	-	-	-	+	-	-
A/Texas/23/12	1.8×10^{1}	1.5×10^{0}	+	-	-	-	+	+	-
A/Washington/29/2009	1.8×10^{1}	1.5×10^{0}	+	-	-	-	+	+	-
A/Texas/48/2009	1.8×10^{1}	1.5×10^{0}	+	-	-	-	+	+	-
A/North Carolina/39/2009	1.8×10^{1}	1.5×10^{0}	+	-	-	-	+	+	-
A/Paraguay/61/2009	6×10^{0}	2.4×10^{0}	+	+	-	-	-	-	-
A/New Caledonia/20/99	6×10^{0}	2.4×10^{0}	+	+	-	-	-	-	-
A/Taiwan/42/06	6×10^{0}	2.4×10^{0}	+	+	-	-	-	-	-

Strain	Sample Type/ Volume								
	200 µL VTM $TCID_{50}$/mL	500 µL VTM $TCID_{50}$/mL	FLUA	AH1	AH3	FLU B	2009 H1	H275 Y	RSV
A/PR/8/34	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/Hong Kong/2652/2006	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/NWS/33	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/Fort Monmouth/1/47	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/Brisbane/59/07	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/New Jersey/8/76	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/Denver/1/57	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/Hawaii/15/2001	6×10^0	2.4×10^0	+	+	-	-	-	-	-
A/Perth/16/09	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Texas/50/12	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Victoria/361/11	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Aichi/2/68	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Port Chalmers/1/73	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Brisbane/10/07	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Wisconsin/67/05	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/New York/55/2004	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Santiago/7981/2006	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Texas/71/2007	2.8×10^0	1.1×10^0	+	-	+	-	-	-	-
A/Minnesota/11/RNA (H3N2v)	$\leq 1 pg/\mu L^{(1)}$	$\leq 1 pg/\mu L^{(1)}$	+	-	+	-	-	-	-
A/Minnesota/10/RNA (H3N2v)	$\leq 1 pg/\mu L^{(1)}$	$\leq 1 pg/\mu L^{(1)}$	+	-	+	-	-	-	-
A/Indiana/10 (H3N2v)	$\leq 1 pg/\mu L^{(1)}$	$\leq 1 pg/\mu L^{(1)}$	+	-	+	-	-	-	-
A/Indiana/8 (H3N2v)	$\leq 1 pg/\mu L^{(1)}$	$\leq 1 pg/\mu L^{(1)}$	+	-	+	-	-	-	-
A/Hubei/1/2010 (H5N1)	$\leq 1 pg/\mu L^{(1)}$	$\leq 1 pg/\mu L^{(1)}$	+	-	-	-	-	-	-
A/Anhui/01/2005 (H5N1)	$\leq 1 pg/\mu L^{(1)}$	$\leq 1 pg/\mu L^{(1)}$	+	-	-	-	-	-	-

Strain	Sample Type/ Volume								
	200 µL VTM $TCID_{50}$/mL	500 µL VTM $TCID_{50}$/mL	FLUA	AH1	AH3	FLU B	2009 H1	H275 Y	RSV
A/India/NIV/2006 (H5N1)	[1] ≤1pg/µL	[1] ≤1pg/µL	+	-	-	-	-	-	-
A/Pheasant/NJ/1355/ 1998 (H5N2)	[1] ≤1pg/µL	[1] ≤1pg/µL	+	-	-	-	-	-	-
A/Turkey/Virginia/2 002 (H7N2)	[1] ≤1pg/µL	[1] ≤1pg/µL	+	-	-	-	-	-	-
A/Mallard/Netherlan ds/12/2000 (H7N7)	[1] ≤1pg/µL	[1] ≤1pg/µL	+	-	-	-	-	-	-
A/Hong Kong/33982/2009 (H9N2)	[1] ≤1pg/µL	[1] ≤1pg/µL	+	-	-	-	-	-	-
A/Chicken/Hong Kong/G9/1997 (H9N2)	[1] ≤1pg/µL	[1] ≤1pg/µL	+	-	-	-	-	-	-
B/Florida/02/06	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Malaysia/2506/04	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Maryland/1/59	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Allen/45	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Hong Kong/5/72	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Taiwan/2/62	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/GL/1739/54	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Lee/40	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
B/Brisbane/60/08	2.8×10^{-1}	1.12×10^{-1}	-	-	-	+	-	-	-
RSVA 2006 Clinical Isolate	1.0×10^{1}	4.0×10^{0}	-	-	-	-	-	-	+
RSVA/A2	1.0×10^{1}	4.0×10^{0}	-	-	-	-	-	-	+
RSVA/Long	[3] 1.0×10^{1}	[3] 4.0×10^{0}	-	-	-	-	-	-	+
RSVB/18537	1.7×10^{-1}	3.4×10^{-1}	-	-	-	-	-	-	+
RSVB/CH93(18)-18	1.7×10^{-1}	3.4×10^{-1}	-	-	-	-	-	-	+
RSVB/WV/14617/8 5	[4] 1.7×10^{0}	3.4×10^{0}	-	-	-	-	-	-	+

Strain	Sample Type/ Volume								
	200 µL VTM $TCID_{50}$/mL	500 µL VTM $TCID_{50}$/mL	FLUA	AH1	AH3	FLU B	2009 H1	H275 Y	RSV

(1) Testing was completed using purified RNA stocks due to biosafety regulations. RNA was serially diluted to an approximately equivalent Cq to the established LoD for Influenza A and tested in triplicate.
(2) The performance characteristics of this device with clinical specimens that are positive for novel avian influenza viruses have not been established.
(3) PFU/mL
(4) 1/3 Replicates failed for RSVB/WV/14617/85 at 1.7 x 10^{-1}. Testing was repeated in triplicate 1 log higher and reported at 1.7 x 10^{0} $TCID_{50}$/mL.

f. Analytical specificity:

To determine the analytical specificity of the IFV-RSV, 51 commensal and pathogenic microorganisms (23 bacteria, 28 viruses) that may be present in the nasal cavity or nasopharynx were tested. All of the following microorganisms were negative when tested at concentrations ranging from 10^6 cells/mL or CFU/mL (bacteria) and 10^5 $TCID_{50}$/mL or PFU/mL (viruses).

Table 10: IFV-RSV Analytical Specificity

Viruses	Bacteria
Adenovirus Type 1	*Bordetella pertussis* A639
Adenovirus Type 3	*Bordetella pertussis* E431
Adenovirus Type 31	*Chlamydia pneumoniae*
Adenovirus Type 4	*Corynebacterium diphtheriae* Z116
Adenovirus Type 5	*Escherichia coli* clinical isolate
Adenovirus Type 7A	*Haemophilus influenzae* type b; MinnA
Coronavirus Strain 229E	*Lactobacillus acidophilus* Z048
Coronavirus Strain NL63	*Lactobacillus plantarum* 17-5
Coronavirus strain OC43	*Legionella longbeachae* Long Beach 4
Coxsackievirus Type A01	*Legionella pneumophila* Philadelphia
Cytomegalovirus Strain AD-169	*Mycoplasma pneumoniae* M129
Epstein Barr Virus (EBV)	*Neisseria meningitidis* serotype A
Enterovirus Type 71 (2003 isoloate)	*Neisseria mucosa* Z060
Human Metapneumovirus (hMPV) 16 Type A1	*Pseudomonas aeruginosa* clinical isolate
Human Metapneumovirus (hMPV) 20 Type A2	*Staphylococcus aureus* MRSA; COL
Human Metapneumovirus 3 (hMPV) Type B1	*Staphylococcus aureus* Z020
Human Metapneumovirus 4 (hMPV) Type B2	*Staphylococcus epidermidis* MRSE; RP62A
Human Metapneumovirus 5 (hMPV) Type B1	*Staphylococcus epidermidis* MSSE; HER 1292

Human Metapneumovirus 8 (hMPV) Type B2	*Streptococcus pneumoniae* 19F; Z022
Human Metapneumovirus 9 (hMPV) Type A1	*Streptococcus pyogenes* Z018
Measles	*Streptococcus salivarius* Z127
Mumps	*Moraxella catarrhalis* Ne 11
Parainfluenza Virus Type 1	*Neisseria sicca* Z043
Parainfluenza Virus Type 2	
Parainfluenza Virus Type 3	
Parainfluenza Virus Type 4A	
Parainfluenza Virus Type 4B	
Rhinovirus Type 1A	

g. Potentially Interfering Substances:

An analytical study was performed to assess the potential interference effects of 21 substances naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity/nasopharynx.

Table 11: IFV-RSV Interfering Substances Tested

Substance	Concentration
Mucin	0.1 mg/mL
Whole Blood	5% v/v
Phenylephrine	10 mg/ml
Oxzymetazoline	10% v/v
Menthol	0.5 mg/mL
Tobramycin	0.15 mg/mL
Histaminum Hydrochloricum	115 µg /ml
NaCl	10% v/v
Luffa Opperculata	1.0% v/v
Sulphur	4.5 mg/ml
Mupirocin	5 µg /ml
Galphimia Glauca	115 µg /ml
FluMist influenza Vaccine, Live*	N/A
Zanamivir	3 mg/ml
Benzocaine	0.5 mg/ml
Beclomethasone dipropionate	16 µg/mL
Dexamethasone	50 µg/mL
Flunisolide	58 µg/mL
Triamcinolone acetonide	5.5 µg/mL
Bedesonide	25 µg/mL
Mometasone Furoate	2.5 µg/mL
Fluticasone propionate	5 µg/mL

*FluMist was not tested as it was not available in the market at the time of testing. The Advisory Committee on Immunization Practices (ACIP) recommended that FluMist not be used for the 2016-2017 season.

The potentially interfering substances were evaluated in the presence of viral targets spiked near 2x LoD consisting of Influenza A (all subtypes detected in IFV), Influenza B and RSV A/B. No evidence of interference caused by these substances was found.

Each interfering substance was tested in triplicate using VTM samples containing 5x LoD of each target at a 500 µL sample volume. To create the interferent test sample, positive simulated specimens were created at 10x LoD, and spiked into VTM media containing the background matrix. For each interferent, the final concentration was obtained by combining a 2x concentration of the interfering substance with 250 µL of the concentrated 10x LoD panel into a final 500 µL volume, resulting in the final 5x LoD concentration of each analyte and the reported final interferent concentration. Each interferent was diluted in the VTM sample containing 5x LoD of each target and tested in triplicate.

h. Competitive Interference:

The competitive interference study evaluated the effects of clinically relevant co-infections with each of the analytes in the IFV-RST test. The study assessed whether a high concentration of one virus (3 logs > LoD) in the specimen could potentially affect the IFV-RSV Panel performance when detecting another target present at low levels in the multiplex Test (approximately 3x LoD). All testing was performed in triplicate. The presence of any virus at high copy number and at LoD in the same Cartridge had no effect on the analytical sensitivity (limit of detection) of the IFV-RSV Panel.

i. Carry-over:

Not applicable; all test components are single use and no reagents come into contact with the instrument.

j. Assay cut-off:

The initial assay cutoff was determined by testing a population of known influenza and respiratory syncytial virus positive and negative samples. A “curve classification” method was then used (Ct offset settings applied to curve analysis) to analyze each PCR curve and determine if the target was detected by finding an exponential growth curve; or else the target was not detected. Next, human experts analyzed each curve in a blinded setting, with the experts reporting curves as positive or negative. The final algorithm was designed to maximize the IFV-RSV Panel LoD while maintaining the assay’s specificity. Clinical samples of known influenza and RSV status (n=204) were tested to determine the percent agreement to Culture or NAT method. The PPA and NPA were calculated and the results support that the test correctly identifies the target analytes.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Not applicable. Performance of the IFV-RSV was evaluated against the reference method in a prospective clinical study.

   b. *Matrix comparison:*

      Not applicable.

3. Clinical Studies:

   The prospective study consisted of freshly collected specimens obtained during the 2015-2016 season (n=214) as well as frozen prospective samples collected during previous seasons (2012-2013 n=494; and 2013-2014 n=306). A total of 1014 prospective samples were collected for use in the clinical study. Of these 1014 samples, fifteen were excluded for protocol deviations or lack of a comparator result, leaving 999 specimens. Since multiple specimen types were collected, not every patient was able to supply all the specimens intended for the study. For 200 µl NPS specimens in VTM, 986 were available for testing. For 500 µl NPS specimens in VTM, 956 were available for testing. There were 26 and 27 QC failures for NPS 200 µl and NP 500 µl specimens, respectively leaving final evaluable specimens of n=960 (NPS 200 µl) and n=929 (NPS 500 µl). The invalid rate during the clinical study was 2.9%.

Table 12: Clinical Study Participant Demographics

Age Group (Years)	Number of Subjects	Proportion (%)
0-2	348	34.3
3-5	143	14.1
6-11	165	16.3
12-18	84	8.3
19-64	249	24.6
>64	25	2.5
All Ages	1014	100

Tables 13 and 14: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A compared to a Molecular Comparator – Prospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	142	3[a]	145
Negative	12[b]	803	815
Total	154	806	960
		Point Estimate	**95% CI**
Positive Percent Agreement		92.2%	86.9% - 95.5%
Negative Percent Agreement		99.6%	98.9% – 99.9%

a: Three samples were FluA positive by Idylla @ 200 µl but negative by the comparator. Sequencing results confirmed one of three samples as FluA positive and two samples as FluA negative.
b: Twelve samples were FluA negative by Idylla @ 200 µl but FluA positive by the comparator. Sequencing results confirmed ten samples as FluA negative and two samples as FluA positive.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	139	5[a]	144
Negative	10[b]	775	785
Total	149	780	929
		Point Estimate	**95% CI**
Positive Percent Agreement		93.3%	88.1% - 96.3%
Negative Percent Agreement		99.4%	98.5% - 99.7%

a: Five samples were FluA positive by Idylla @ 500 µl but negative by the comparator. Sequencing results confirmed four samples as FluA negative and one sample as FluA positive.
b: Ten samples were FluA negative by Idylla @ 500 µl but positive by the comparator. Sequencing results confirmed nine samples as FluA negative and one sample as FluA positive.

Tables 15 and 16: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A H1 compared to a Molecular Comparator – Prospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	25	0	25
Negative	1[a]	382	383
Total	26	382	408
		Point Estimate	**95% CI**
Positive Percent Agreement		96.2%	81.1% - 99.3%
Negative Percent Agreement		100%	99.0% – 100%

a: One sample was not tested by sequencing as it was positive at 500 µL

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	25	0	25
Negative	0	393	393
Total	25	393	418
		Point Estimate	**95% CI**
Positive Percent Agreement		100%	86.7% - 100%
Negative Percent Agreement		100%	99.0% - 100%

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Tables 17 and 18: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A H3 compared to a Molecular Comparator – Prospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	73	0	73
Negative	5[a]	882	887
Total	78	882	960
		Point Estimate	**95% CI**
Positive Percent Agreement		93.6%	85.9 – 97.2%
Negative Percent Agreement		100%	99.6% – 100%

a: Five samples were A H3 negative by Idylla @ 200 µL but positive by the comparator. Sequencing confirmed all samples A H3 negative.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	71	1[a]	72
Negative	4[b]	853	857
Total	75	854	929
		Point Estimate	**95% CI**
Positive Percent Agreement		94.7%	87.1% - 97.9%
Negative Percent Agreement		99.9%	99.3% - 100%

a: One sample was AH3 positive by Idylla @ 500 µL but negative by the comparator. Sequencing result confirmed A H3 positive

b: Four samples were A H3 negative by Idylla @ 500 µL but positive by the comparator. Sequencing result confirmed all samples as AH3 negative.

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Tables 19 and 20: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A 2009 H1 compared to a Molecular Comparator – Prospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	66	2[a]	68
Negative	10[b]	882	892
Total	76	884	960
		Point Estimate	**95% CI**
Positive Percent Agreement		86.8%	77.4% - 92.7%
Negative Percent Agreement		99.8%	99.2% – 99.9%

a: Two samples were 2009H1 positive by Idylla @ 200 µl and negative by the comparator. Sequencing results confirmed one sample as 2009H1 positive and one as negative.
b: Ten samples were 2009H1 negative by Idylla @ 200 µl and positive by the comparator. Nine samples were confirmed negative and one sample was confirmed positive by sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	65	2[a]	67
Negative	8[b]	854	862
Total	73	856	929
		Point Estimate	**95% CI**
Positive Percent Agreement		89.0%	79.8% - 94.3%
Negative Percent Agreement		99.8%	99.2% - 99.9%

a: Two samples were 2009H1 positive by Idylla @ 500 µl and negative by the comparator. Sequencing results confirmed one sample as 2009H1 positive and one sample as negative.
b: Eight samples were 2009H1 negative by Idylla @ 500 µl but positive by the comparator. Sequencing results confirmed seven samples as 2009H1 negative and one sample positive.

Tables 21 and 22: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza B compared to a Molecular Comparator – Prospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	113	2[a]	115
Negative	19[b]	826	845
Total	132	828	960
		Point Estimate	**95% CI**
Positive Percent Agreement		85.6%	78.6% - 90.6%
Negative Percent Agreement		99.8%	99.1% – 99.9%

a: Two samples were FluB positive by Idylla but negative by the comparator @ 200 µl. Sequencing result confirmed two samples FluB negative.
b: 19 samples were FluB negative by Idylla but positive by the comparator @ 200 µl. Two samples were confirmed positive by sequencing and 17 samples were confirmed negative by sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	111	2[a]	113
Negative	19[b]	797	816
Total	130	799	929
		Point Estimate	**95% CI**
Positive Percent Agreement		85.4%	78.3% - 90.4%
Negative Percent Agreement		99.7%	99.1% – 99.9%

a: Two samples were FluB positive by Idylla but negative by the comparator @ 500 µl. DNA sequencing confirmed both samples as FluB negative.
b: 19 samples were FluB negative by Idylla but positive by the comparator @ 500 µl. DNA sequencing confirmed three samples as FluB positive and 16 samples as FluB negative.

Tables 23 and 24: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for RSV (A and B) compared to a Molecular Comparator – Prospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	96	3[a]	99
Negative	14[b]	847	861
Total	110	850	960
		Point Estimate	**95% CI**
Positive Percent Agreement		90.2%	85.0% - 94.0%
Negative Percent Agreement		99.7%	99.1% – 99.9%

a: Three samples were RSV positive by Idylla @ 200 µl but RSV negative by the comparator. Two samples were confirmed negative and one sample was confirmed negative by sequencing.
b: 14 samples were RSV negative by Idylla @ 200 µl but RSV positive by the comparator. Four samples were confirmed RSV negative by sequencing. 10 samples were confirmed RSV positive by sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	90	3[a]	93
Negative	13[b]	823	836
Total	103	826	929
		Point Estimate	**95% CI**
Positive Percent Agreement		87.4%	79.6% - 92.4%
Negative Percent Agreement		99.6%	98.9% – 99.9%

a: Three samples were RSV positive by Idylla but negative by the comparator @ 500 µl. Sequencing results confirmed one RSV positive and two RSV negative samples.
b: 13 samples were RSV negative @ 500 µl by Idylla and RSV positive by the comparator. Sequencing results confirmed four samples as RSV negative and nine samples as RSV positive.

A separate study was performed using nasopharyngeal swab specimens from archived banked retrospective clinical collections tested on the Idylla Respiratory (IFV RSV) Panel and comparator Assays. Testing of the IFV-RSV Panel was conducted by three operators on the three Idylla Systems for both 200 µl and 500 µl samples. A total of 419 patient samples were tested at 200 µl with 408 reported results, (invalid rate of 1%) and a

total of 449 patient samples were tested at 500 µl with 418 reported results (invalid rate of 7%). The results for the retrospective testing for each target, including Positive and Negative Agreement for the comparison study, are presented in the following tables. The IFV-RSV Panel performance was compared to an FDA-cleared Nucleic Acid Amplification Test (NAAT).

Tables 25 and 26: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A compared to a Molecular Comparator – Retrospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	80	0	80
Negative	4[a]	324	328
Total	84	324	408
		Point Estimate	**95% CI**
Positive Percent Agreement		95.2%	88.4% - 98.1%
Negative Percent Agreement		100%	98.9% – 100%

a: Four samples were FluA negative by Idylla @ 200ul but positive by the comparator. One sample was negative at 200ul by Idylla but detected at 500ul by Idylla; therefore the sample was not tested in sequencing. Two samples were confirmed FluA negative by sequencing. One of the sample had insufficient volume remained to complete sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	82	0	82
Negative	3[a]	333	336
Total	85	333	418
		Point Estimate	**95% CI**
Positive Percent Agreement		96.5%	90.1% - 98.8%
Negative Percent Agreement		100%	98.9% – 100%

a: Three samples were FluA negative by Idylla @ 500ul but positive by the comparator. Two of the three samples were confirmed FluA negative by sequencing. One sample had insufficient volume to complete DNA sequencing.

Tables 27 and 28: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A H1 compared to a Molecular Comparator – Retrospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	25	0	25
Negative	1[a]	382	383
Total	26	382	408
		Point Estimate	**95% CI**
Positive Percent Agreement		96.2%	81.1% - 99.3%
Negative Percent Agreement		100%	99.0% – 100%

a: One sample was Idylla negative for AH1 at 200uL but detected by Idylla at 500uL, therefore not tested in sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	25	0	25
Negative	0	393	393
Total	25	393	418
		Point Estimate	**95% CI**
Positive Percent Agreement		100%	86.7% - 100%
Negative Percent Agreement		100%	99.0% - 100%

Tables 29 and 30: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A H3 compared to a Molecular Comparator – Retrospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	27	0	27
Negative	1[a]	380	381
Total	28	380	408
		Point Estimate	**95% CI**
Positive Percent Agreement		96.4%	82.3% - 99.4%
Negative Percent Agreement		100%	99.0% – 100%

a: One sample was negative for AH3 by Idylla @ 200ul but the comparator result was AH3 positive. Insufficient material remained for DNA Sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	28	0	28
Negative	1[a]	389	390
Total	29	389	418
		Point Estimate	**95% CI**
Positive Percent Agreement		96.6%	82.8% - 99.4%
Negative Percent Agreement		100%	99.0% - 100%

a: One sample was negative for AH3 by Idylla @ 500ul but the comparator result was AH3 positive. Insufficient material remained for DNA Sequencing.

Tables 31 and 32: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A 2009 H1 compared to a Molecular Comparator– Retrospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	28	0	28
Negative	1[a]	379	380
Total	29	379	408
		Point Estimate	**95% CI**
Positive Percent Agreement		96.6%	82.8% - 99.4%
Negative Percent Agreement		100%	99.0% – 100%

a: One sample was 2009H1 negative at 200uL by Idylla, but detected by the comparator. DNA sequencing confirmed the absence of H1N1 2009 and Influenza A.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	29	0	29
Negative	1[a]	388	389
Total	30	388	418
		Point Estimate	**95% CI**
Positive Percent Agreement		96.7%	83.3% - 99.4%
Negative Percent Agreement		100%	99.0% - 100%

a: One sample was 2009H1 negative at 500uL by Idylla, but detected by the comparator. DNA sequencing confirmed the absence of H1N1 2009 and Influenza A.

Tables 33 and 34: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza B compared to a Molecular Comparator – Retrospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	102	1[a]	103
Negative	5[b]	300	305
Total	107	301	408
		Point Estimate	**95% CI**
Positive Percent Agreement		95.3%	89.5% - 98.0%
Negative Percent Agreement		99.7%	98.1% – 100%

a: One sample was FluB positive by Idylla @ 200ul but negative by the comparator. This sample was confirmed FluB positive by DNA sequencing.
b: Five samples were FluB negative by Idylla @ 200ul and positive by the comparator. All five samples were confirmed FluB positive by sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	106	1[a]	107
Negative	6[b]	305	311
Total	112	306	418
		Point Estimate	**95% CI**
Positive Percent Agreement		94.6%	88.8% - 97.5%
Negative Percent Agreement		99.7%	98.2% – 99.9%

a: One sample was FluB positive by Idylla but negative by the comparator @ 500ul. DNA sequencing confirmed this sample as FluB positive.
b: Six samples were FluB negative by Idylla @ 500 ul but positive by the comparator. All six samples were confirmed FluB positive by DNA sequencing.

Tables 35 and 36: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for RSV (A and B) compared to a Molecular Comparator – Retrospective Specimens

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	69	1 [a]	70
Negative	4 [b]	334	338
Total	73	335	408
		Point Estimate	**95% CI**
Positive Percent Agreement		94.5%	86.7% - 97.9%
Negative Percent Agreement		99.7%	98.3% – 100%

a: One sample was RSV positive by Idylla @ 200ul but negative by the comparator. Insufficient sample remained for DNA sequencing.
b: Four samples were RSV negative by Idylla @ 200ul but positive by the comparator. All four samples were confirmed negative by sequencing.

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	81	1 [a]	82
Negative	7 [b]	329	336
Total	88	330	418
		Point Estimate	**95% CI**
Positive Percent Agreement		92.1%	84.5% - 96.1%
Negative Percent Agreement		99.7%	98.3% – 100%

a: One RSV sample was positive by Idylla @500ul but negative by the comparator. Sequencing result confirmed this sample as RSV positive.
b: Seven samples were RSV negative by Idylla @ 500ul but the comparator. Six samples were confirmed RSV negative and one sample confirmed RSV positive by sequencing.

Testing of 40 contrived samples containing Influenza A virus with the H275Y mutation was performed by introducing the contrived samples into the frozen sample testing workflow. Each sample returned a positive result by the comparator method for Influenza A H1 2009 and a negative result for all other targets. These samples were then tested for the H275Y mutation using a validated bidirectional sequencing assay. Each of these returned a positive H275Y mutation result from the bidirectional sequencing assay. Using

the IFV–RSV assay to test the same 40 contrived specimens, each of the valid test results was positive for Influenza A H1 2009 and the H275Y mutation

Tables 37 and 38: IFV-RSV Positive Percent Agreement and Negative Percent Agreement for Influenza A 2009 H1 Genotype H275Y compared to Sequencing

200 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	39	0	39
Negative	0	960	960
Total	39	960	999
		Point Estimate	**95% CI**
Positive Percent Agreement		100%	91.0% - 100%
Negative Percent Agreement		100%	99.6% – 100%

500 µl VTM			
IFV-RSV Panel Result	**Comparator Positive**	**Comparator Negative**	**Total**
Positive	40	0	40
Negative	0	929	929
Total	40	929	969
		Point Estimate	**95% CI**
Positive Percent Agreement		100%	91.2% – 100%
Negative Percent Agreement		100%	99.6% – 100%

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   In the IFV-RSV prospective clinical study (described in the "Clinical Studies" section above), a total of 960 NPS 200 µl and 929 NPS 500 µl specimens were evaluable by the IFV-RSV assay. The number and percentage of analyte positive cases per specified age group, as determined by the IFV-RSV assay, are presented in the tables below for each specimen type:

Table 39: IFV-RSV Expected Values for 200 µl Specimens

Age	200 µL: 2012-2013 season				200 µL: 2013-2014 season				200 µL: 2015-2016 season		
	FLU A				FLU A				FLU A		
	N	Positive	Prevalence		N	Positive	Prevalence		N	Positive	Prevalence
0-2	137	13	9.5%		124	9	7.3%		63	11	17.5%
3-5	66	5	7.6%		42	7	16.7%		30	4	13.3%
6-11	94	11	11.7%		45	4	8.9%		18	2	11.1%
12-18	44	8	18.2%		25	6	24.0%		11	3	27.3%
19-64	106	16	15.1%		60	20	33.3%		72	22	30.6%
>64	13	3	23.1%		6	0	0.0%		7	3	42.9%
	FLU AH3				FLU AH3				FLU AH3		
0-2	137	13	9.5%		124	2	1.6%		63	0	0.0%
3-5	66	5	7.6%		42	3	7.1%		30	1	3.3%
6-11	94	10	10.6%		45	1	2.2%		18	0	0.0%
12-18	44	7	15.9%		25	3	12.0%		11	0	0.0%
19-64	106	13	12.3%		60	12	20.0%		72	1	1.4%
>64	13	3	23.1%		6	0	0.0%		7	0	0.0%
	FLU A 2009H1				FLU A 2009H1				FLU A 2009H1		
0-2	137	0	0.0%		124	7	5.6%		63	11	17.5%
3-5	66	0	0.0%		42	4	9.5%		30	2	6.7%
6-11	94	1	1.1%		45	3	6.7%		18	1	5.6%
12-18	44	0	0.0%		25	3	12.0%		11	3	27.3%
19-64	106	3	2.8%		60	9	15.0%		72	19	26.4%
>64	13	0	0.0%		6	0	0.0%		7	3	42.9%
	FLU B				FLU B				FLU B		
0-2	137	10	7.3%		124	0	0.0%		63	9	14.3%
3-5	66	18	27.3%		42	1	2.4%		30	10	33.3%
6-11	94	28	29.8%		45	0	0.0%		18	6	33.3%
12-18	44	8	18.2%		25	2	8.0%		11	2	18.2%
19-64	106	13	12.3%		60	1	1.7%		72	6	8.3%
>64	13	1	7.7%		6	0	0.0%		7	0	0.0%
	RSV				RSV				RSV		
0-2	137	21	15.3%		124	34	27.4%		63	10	15.9%
3-5	66	0	0.0%		42	9	21.4%		30	6	20.0%
6-11	94	1	1.1%		45	2	4.4%		18	0	0.0%
12-18	44	1	2.3%		25	1	4.0%		11	0	0.0%
19-64	106	7	6.6%		60	3	5.0%		72	2	2.8%
>64	13	2	15.4%		6	1	16.7%		7	0	0.0%

*No positive prospective samples were detected for non-2009 Influenza A H1, or the H275Y genotype of Influenza A 2009 H1 thus prevalence for those viruses was zero.

Table 40: IFV-RSV Expected Values for 500 µl Specimens

	500 µL: 2012-2013 season				500 µL: 2013-2014 season				500 µL: 2015-2016 season		
	FLU A				FLU A				FLU A		
AGE	N	Positive	Prevalence		N	Positive	Prevalence		N	Positive	Prevalence
0-2	130	14	10.8%		120	9	7.5%		59	11	18.6%
3-5	62	4	6.5%		42	7	16.7%		27	3	11.1%
6-11	89	10	11.2%		45	4	8.9%		18	2	11.1%
12-18	42	8	19.0%		24	5	20.8%		11	4	36.4%
19-64	105	17	16.2%		60	20	33.3%		72	22	30.6%
>64	13	3	23.1%		6	0	0.0%		7	3	42.9%
	FLU AH3				FLU AH3				FLU AH3		
0-2	130	13	10.0%		120	2	1.7%		59	0	0.0%
3-5	62	4	6.5%		42	3	7.1%		27	1	3.7%
6-11	89	9	10.1%		45	1	2.2%		18	0	0.0%
12-18	42	8	19.0%		24	3	12.5%		11	0	0.0%
19-64	105	14	13.3%		60	12	20.0%		72	0	0.0%
>64	13	3	23.1%		6	0	0.0%		7	0	0.0%
	FLU A 2009H1				FLU A 2009H1				FLU A 2009H1		
0-2	130	0	0.0%		120	7	5.8%		59	11	18.6%
3-5	62	0	0.0%		42	4	9.5%		27	2	7.4%
6-11	89	0	0.0%		45	3	6.7%		18	1	5.6%
12-18	42	0	0.0%		24	2	8.3%		11	3	27.3%
19-64	105	3	2.9%		60	9	15.0%		72	20	27.8%
>64	13	0	0.0%		6	0	0.0%		7	3	42.9%
	FLU B				FLU B				FLU B		
0-2	130	10	7.7%		120	0	0.0%		59	9	15.3%
3-5	62	17	27.4%		42	1	2.4%		27	10	37.0%
6-11	89	27	30.3%		45	0	0.0%		18	7	38.9%
12-18	42	9	21.4%		24	2	8.3%		11	2	18.2%
19-64	105	12	11.4%		60	1	1.7%		72	5	6.9%
>64	13	1	7.7%		6	0	0.0%		7	0	0.0%
	RSV				RSV				RSV		
0-2	130	21	16.2%		120	34	28.3%		59	7	11.9%
3-5	62	0	0.0%		42	9	21.4%		27	5	18.5%
6-11	89	1	1.1%		45	2	4.4%		18	0	0.0%
12-18	42	0	0.0%		24	1	4.2%		11	0	0.0%
19-64	105	5	4.8%		60	4	6.7%		72	2	2.8%
>64	13	2	15.4%		6	1	16.7%		7	0	0.0%

*No positive prospective samples were detected for non-2009 Influenza A H1, or the H275Y genotype of Influenza A 2009 H1 thus prevalence for those viruses was zero.

N. Instrument Name:

Idylla Instrument

O. System Descriptions:

1. Modes of Operation:

   The Idylla Respiratory Panel is a self-contained molecular *in vitro* diagnostic test designed to work with the Idylla System. The Idylla System consists of the Idylla console connected to between one and eight Idylla Instruments, and the test cartridge. The Idylla Instrument's function is the automated processing of Idylla Test specific cartridges. Processing is triggered by the user loading a cartridge into the instrument. Upon loading of a cartridge into the Instrument, the Instrument automatically processes the test specific cartridges according to the corresponding Test specific software that provides instructions for processing of PCR fluorescence data into reportable results. The Instrument's front panel acts as the Instrument's user interface. It has a button to open and close the cartridge tray, and it uses LEDs (indicator lamps) to indicate the status of the Instrument.

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes _____ or No ____X____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

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:

   Specimen ID is entered manually using a keyboard or by a barcode scanner.

4. Specimen Sampling and Handling:

   Not applicable. The specimens are manually inserted in the cartridge before being placed in the instrument.

5. Calibration:

   The Idylla Instrument is factory calibrated and does not require any further calibration and verification at user site. External controls are recommended to ensure proper functionality by the manufacture.

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

Not applicable.

Q. Proposed Labeling:

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

R. Conclusion:

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