



Food and Drug Administration
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Silver Spring, MD 20993-0002

Luminex Corporation
Wendy Ricker
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Austin TX 78727

August 2, 2016

Re: K161220
Trade/Device Name: ARIES[®] Flu A/B & RSV Assay
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: II
Product Code: OCC, OOI, OZE
Dated: April 29, 2016
Received: April 29, 2016

Dear Ms. Ricker:

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

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>. Also, please note the regulation entitled, “Misbranding by reference to premarket notification” (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH’s Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

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

Sincerely yours,

Tamara V. Feldblyum -S for

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

Enclosure

Indications for Use

510(k) Number (if known)
K161220

Device Name

ARIES[®] Flu A/B & RSV Assay

Indications for Use (Describe)

The ARIES[®] Flu A/B & RSV Assay is a polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the direct detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) nucleic acid in nasopharyngeal swab (NPS) specimens from patients with signs and symptoms of respiratory tract infection in conjunction with clinical and laboratory findings. The test is intended for use as an aid in the differential diagnosis of Influenza A, Influenza B, and RSV 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 diagnosis, treatment or other 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 (e.g. bacterial culture, immunofluorescence, X-ray findings) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory viral infection.

Performance characteristics for influenza A were established during the 2014-2015 and the 2015-2016 influenza seasons when influenza A/H3N2 and A/H1N1 pandemic 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 BSL 3+ facility is available to receive and culture specimens.

The ARIES[®] Flu A/B & RSV Assay is indicated for use with the ARIES[®] Systems.

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

Prescription Use (Part 21 CFR 801 Subpart D)

Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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11.0 Executive Summary 510(k)

This Executive Summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

A. 510(k) Number:

K161220

B. Purpose for Submission:

Traditional 510(k), New Device

C. Measurand:

Targets RNA sequences for the conserved regions of the matrix protein genes of influenza A and influenza B viruses, and the fusion gene of Respiratory Syncytial Virus (RSV).

D. Type of Test:

Qualitative Real Time Polymerase Chain Reaction (PCR)

E. Applicant:

Luminex Corporation

F. Proprietary and Established Names:

ARIES® Flu A/B & RSV Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OCC	II	21 CFR 866.3980—Respiratory viral panel multiplex nucleic acid assay	Microbiology (83)

H. Intended Use:1. Intended use(s):

The ARIES® Flu A/B & RSV Assay is a polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the direct detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) nucleic acid in nasopharyngeal swabs (NPS) specimens from patients with signs and symptoms of respiratory tract infection in conjunction with clinical and laboratory findings. The test is intended for use as an aid in the differential diagnosis of Influenza A, Influenza B, and RSV 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 diagnosis, treatment or other 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 (e.g. bacterial culture, immunofluorescence, X-ray findings) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory viral infection.

Performance characteristics for influenza A were established during the 2014-2015 and the 2015-2016 influenza seasons when influenza A/H3N2 and A/H1N1 pandemic 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 BSL 3+ facility is available to receive and culture specimens.

The ARIES® Flu A/B & RSV Assay is indicated for use with the ARIES® Systems.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

For use with the ARIES® System or ARIES® M1 System.

I. **Device Description:**

The ARIES® Flu A/B & RSV Assay is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test system that will consist of an ARIES® System with its included software, an assay-specific cassette, and an assay-specific protocol file. The ARIES® Flu A/B & RSV Assay cassette is a disposable, single-use cassette containing nucleic acid purification reagents, internal sample process control (SPC), and an assay-specific master mix capable of performing the designated assay on one sample. The ARIES® Flu A/B & RSV Assay cassette directly detects and differentiates influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) nucleic acid in nasopharyngeal swabs (NPS) specimens from patients with signs and symptoms of respiratory tract infection in conjunction with clinical and laboratory findings. Specifically, the ARIES® Flu A/B & RSV Assay cassette detects the matrix protein genes of influenza A and influenza B viruses, and the fusion gene of RSV and a RNA Sample Processing Control.

The specimen is lysed and nucleic acid is extracted using an ARIES® System. An extractable sample processing control (SPC) target is present in the ARIES® Flu A/B & RSV assay cassette and is processed with the specimen. The SPC controls for specimen lysis, for recovery of extracted nucleic acid, for inhibitory substances and for PCR reagent and instrument integrity. The Ct value of the SPC is designed to verify proper specimen lysis and nucleic acid extraction, to identify PCR inhibition, if any, and verify proper function of the extraction system and real-time instrument. The Tm value of the SPC is used as a reference for determining the target Tm.

The extracted nucleic acid and SPC are transferred via magnetic beads through the cassette to the ARIES® Flu A/B & RSV Assay lyophilized PCR reagents in the PCR tube that contain primer pairs specific to influenza A, influenza B, RSV, and the SPC sequence. The specific primer pairs are labeled with distinct fluorophore labels. PCR amplification is performed and assay fluorescence is monitored on an ARIES® System. Incorporation of the quencher-labeled nucleotide causes a decrease in assay fluorescence. Following amplification, the reaction is slowly heated and fluorescence is monitored. The strands of the amplification products will separate at a specific melting temperature (Tm) that is determined by an increase in fluorescence as the strands are separated. The instrument fluorescence output is analyzed and test results are determined using the ARIES® Flu A/B & RSV Assay protocol file. A printed results report is generated.

J. **Substantial Equivalence Information:**

1. Predicate device name(s):

Simplexa Flu A/B & RSV Direct, Simplexa Flu A/B & RSV Positive Control Pack (manufactured by Focus Diagnostics, Inc.)

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

K120413

3. Comparison with predicate:

The following table compares the ARIES® Flu A/B & RSV Assay to Focus’ Simplexa Flu A/B & RSV Direct, Simplexa Flu A/B & RSV Positive Control Pack (k120413). Table 11.1 shows similarities between the new device and the predicate, while Table 11.2 shows the differences.

Table 11.1: Similarities between New Device and Predicate

Similarities		
Attribute	New Device	Predicate Device (K120413)
Intended Use	<p>The ARIES® Flu A/B & RSV Assay is a polymerase chain reaction (PCR) based qualitative <i>in vitro</i> diagnostic test for the direct detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) nucleic acid in nasopharyngeal swabs (NPS) specimens from patients with signs and symptoms of respiratory tract infection in conjunction with clinical and laboratory findings. The test is intended for use as an aid in the differential diagnosis of Influenza A, Influenza B, and RSV in humans and is not intended to detect Influenza C.</p> <p>Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Conversely, positive results do not rule-out bacterial infection or co-infection with other viruses.</p> <p>The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial culture, immunofluorescence,</p>	<p>The Focus Diagnostics Simplexa™ Flu A/B & RSV Direct assay is intended for use on the 3M Integrated Cycler instrument for the <i>in vitro</i> 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.</p> <p>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.</p> <p>Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009</p>

	<p>X-ray findings) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory viral infection.</p> <p>Performance characteristics for influenza A were established during the 2014-2015 and the 2015-2016 influenza seasons when influenza A/H3N2 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary.</p> <p>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 BSL 3+ facility is available to receive and culture specimens.</p> <p>The ARIES® Flu A/B & RSV Assay is indicated for use with the ARIES® Systems.</p>	<p>H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.</p> <p>If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.</p>
Assay Targets	RNA from Influenza A, Influenza B, and RSV	RNA from Influenza A, Influenza B, and RSV
Influenza A Target	Matrix gene	Matrix gene
Influenza B Target	Matrix gene	Matrix gene
Sample type	Nasopharyngeal swabs (NPS)	Nasopharyngeal swabs (NPS)
Assay format	Real-time PCR	Real-time PCR
Assay results	Qualitative	Qualitative

Table 11.2: Differences between New Device and Predicate

Differences		
Attribute	New Device	Predicate Device (K120413)
Extraction Method	Automated by an ARIES® System	No extraction
RSV Target	Fusion gene	M gene
Controls	Sample processing control	RNA Internal Control, Positive Controls Sold Separately
Instrument	ARIES® System or ARIES® M1 System	3M™ Integrated Cyclor

K. Standards/Guidance Documents Referenced:

1. Guidance for Industry and FDA Staff, Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay, October 9, 2009
2. Guidance for Industry and FDA Staff - Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses, July 15, 2011
3. Guidance for Industry and FDA Staff, In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path, May 1, 2007.

L. Test Principle:

The ARIES® Flu A/B & RSV Assay chemistry is based on an expanded genetic alphabet technology, consisting of synthetic DNA base pair 2'-deoxy-5-methyl-isocytidine (iC): 2'-deoxyisoguanosine (iG). The isobases (iC and iG) pair specifically with each other and not with natural nucleotides. In addition, isobases are efficiently incorporated during PCR. During PCR amplification, a quencher-modified iGTP is incorporated by the polymerase opposite an iC and a fluorophore reporter attached to a PCR primer. If the target is present and is amplified, assay fluorescence decreases with every cycle as amplification product accumulates. The decrease in assay fluorescence is monitored in real time using an ARIES® System. Following PCR, the amplification products are thermally denatured and assay fluorescence is monitored. The strands of the amplification products are separated and assay fluorescence increases, thus enabling determination of the melting temperature (T_m) of the amplicon.

M. Performance Characteristics:

1. Analytical performance:

- a. *Precision/Reproducibility:*

Precision

Within Laboratory Precision of the ARIES® Flu A/B & RSV Assay was evaluated by two operators performing testing with four ARIES® Systems using three lots of ARIES® Flu A/B & RSV Assay cassettes. Testing was performed on 12 nonconsecutive days and included a total of 675 replicates of a representative reproducibility panel. The reproducibility panel contained influenza A, influenza B, RSV-A, and RSV-B and representative viral cultures were diluted to three concentrations: moderate positive (10x LoD), low positive (3x LoD), and high negative (0.125x LoD for influenza A, and 0.25x LoD for influenza B, RSV-A and RSV-B). All dilutions were prepared in a simulated nasal matrix (SNM); negative samples consisted of only SNM. The overall

invalid percentage for this study was 1.3% (9/675). The results of the reproducibility study are summarized in Table 11-3.

Table 11-3: ARIES® Flu A/B & RSV Assay Within Laboratory Precision Determination Results

Strain	Target Concentration	Expected Positivity	Positivity	95% Confidence Interval
Influenza A/Hong Kong/8/68	Moderate Positive	100%	100% (48/48)	93% - 100%
	Low Positive	Approximately 95%	100% (48/48)	93% - 100%
	High Negative	20% – 80%	79% (38/48)	65% - 90%
Influenza B/Florida/04/06	Moderate Positive	100%	100% (48/48)	93% - 100%
	Low Positive	Approximately 95%	100% (48/48)	93% - 100%
	High Negative	20% – 80%	81% (39/48) ^a	67% - 91%
RSV A2	Moderate Positive	100%	100% (48/48)	93% - 100%
	Low Positive	Approximately 95%	96% (46/48)	86% - 99%
	High Negative	20% – 80%	48% (23/48)	33% - 63%
RSV B WV/14617/85	Moderate Positive	100%	100% (48/48)	93% - 100%
	Low Positive	Approximately 95%	100% (48/48)	93% - 100%
	High Negative	20% – 80%	90% (43/48) ^a	77% - 97%
Flu A/B & RSV Negative	Negative	0%	2.1% (2/96) ^b	0% - 7%

^a RSV B WV/14617/45 and influenza B/Florida/04/06 High Negative samples generated positivity that exceeded the expected positivity results of 20-80%.

^b Two Flu A/B & RSV Negative replicates tested by different operators, on different test dates, on different instruments, with different cassette lots generated late Ct false influenza B Positive results. Overall percentage of negative specimens that correctly generated negative results for all three assay targets is 98%.

Reproducibility

Reproducibility of the ARIES® Flu A/B & RSV Assay was evaluated by testing one lot of ARIES® Flu A/B & RSV Assay Cassettes on two ARIES® Systems by two operators at each of three clinical laboratory sites on five non-consecutive days. A reproducibility panel was prepared containing a moderate positive (10x LoD), low positive (3x LoD), and high negative (0.2x LoD) independently for influenza A, influenza B, RSV-A, and RSV-B, as well as a negative sample. The reproducibility panels were created by an independent operator and blinded to the testing sites. Each panel member was

tested in triplicate by each operator on each day of testing. The results of the reproducibility study are shown in Tables 11-4 and 11-5.

Table 11-4: ARIES® Flu A/B & RSV Assay Site-to-Site Reproducibility Results ^{a,b}

		Site 1		Site 2		Site 3	
		Positivity		Positivity		Positivity	
Influenza A	High Negative	27/30	90.0%	30/30	100.0%	26/30	86.7%
	Low Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%
	Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%
	Negative	1/30	3.3%	0/30	0.0%	0/30	0.0%
Influenza B	High Negative	9/30	30.0%	7/30	23.3%	15/30	50.0%
	Low Positive	29/30	96.7%	30/30	100.0%	30/30	100.0%
	Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%
	Negative	0/30	0.0%	0/30	0.0%	0/30	0.0%
RSV ^c	High Negative	39/60	65.0%	50/60	83.3%	43/60	71.7%
	Low Positive	60/60	100.0%	60/60	100.0%	60/60	100.0%
	Moderate Positive	60/60	100.0%	60/60	100.0%	59/60	98.3%
	Negative	0/30	0.0%	0/30	0.0%	0/30	0.0%

^a An overall invalid rate of 0.9% (11/1260) was observed in the target replicates.

^b The expected result for: a moderate positive target was 100% positive, low positive target was approximately 95% positive, high negative was 20 – 80% positive, and negative was 0% positive.

^c RSV-A and RSV-B are not differentiated by the ARIES Flu A/B & RSV Assay and, therefore, are combined and represented as RSV only.

Table 11-5: Reproducibility Panel Total Results ^{a,b}

		Positivity		95% C.I.	
				lower limit	upper limit
Influenza A	High Negative	83/90	92.2%	84.6%	96.8%
	Low Positive	90/90	100.0%	95.9%	100.0%
	Moderate Positive	90/90	100.0%	95.9%	100.0%
	Negative	1/90	1.1%	1.1%	6.0%
Influenza B	High Negative	31/90	34.4%	24.7%	45.2%
	Low Positive	89/90	98.9%	94.0%	100.0%
	Moderate Positive	90/90	100.0%	95.9%	100.0%
	Negative	0/90	0.0%	0.0%	4.0%
RSV ^c	High Negative	132/180	73.3%	66.2%	79.6%
	Low Positive	180/180	100.0%	95.9%	100.0%
	Moderate Positive	179/180	99.4%	96.9%	100.0%
	Negative	0/90	0.0%	0.0%	4.0%

^a An overall invalid rate of 0.9% (11/1260) was observed in the target replicates.

^b The expected result for: a moderate positive target was 100% positive, low positive target was approximately 95% positive, high negative was 20 – 80% positive, and negative was 0% positive.

^c RSV-A and RSV-B are not differentiated by the ARIES Flu A/B & RSV Assay and, therefore, are combined and represented as RSV only.

b. Linearity/assay reportable range:

Not applicable. The ARIES® Flu A/B & RSV Assay is a qualitative assay.

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

Stability:

Specimen Stability (Fresh vs. Frozen)

Fresh vs. frozen specimen stability was determined with contrived fresh and frozen specimens stored at either 2-8°C (fresh) or -65 to -95°C (frozen) and tested with the ARIES® Flu A/B & RSV Assay. This was assessed by testing 60 replicates for a single target pathogen (influenza A, influenza B, or RSV) prepared at 3 test concentrations (30 low positive, 15 moderate positive, and 15 high positive) across 9 different time points extending out to 12 months. In addition to the 60 replicates for each target organism, 6 replicates of an influenza A/B and RSV negative specimen in simulated nasal matrix (SNM) were tested for each time point extending out to 12 months. Data up to 3 months was collected with all targets yielding the expected result. Overall, high positive specimens were 100% positive, moderate positive specimens were 100% positive, and low positive specimens were positive approximately 95% of the time for all three evaluated targets. Negative specimens were negative 100% of the time. Influenza A, influenza B, and RSV specimens are stable for up to 7 days when stored at 2-8°C and 3 months when stored at -65 to -95°C.

Shelf-Life Stability

Real time stability study was performed to evaluate the shelf life of ARIES® Flu A/B & RSV Assay cassettes. Stability was established by testing 6 replicates of ARIES® Flu A/B & RSV Assay Extractable Control, 100X LOD blend (PN 10188) and negative targets (UTM) on three different lots of ARIES® Flu A/B & RSV Assay cassettes stored at 2 different temperatures: 4°C (2 – 8°C) and room temperature (15 – 30°C) at 10 different time points extending out to 19 months. Acceptance criteria for stability at each time point and temperature was established as 100% positivity for all influenza A/B and RSV replicates, and 100% negativity for all negative replicates. Data collected up to 5 months gave expected results indicating stability of the of ARIES® Flu A/B & RSV Assay cassettes up to 5 months.

Open Box Stability

An Open Box Stability study was performed in order to evaluate performance of ARIES® Flu A/B & RSV Assay Cassettes after they were removed from their individual pouches. Cassettes were removed from their pouches and placed on a laboratory bench where they were exposed to ambient temperatures, humidity, and light for up to ten (10) hours. Data was collected for contrived influenza A, influenza B, and RSV positive and influenza A, influenza B, and RSV negative (Copan Universal Transport Media) samples at five time points throughout a ten (10) hour duration. Three lots of cassettes were used to assess Open Box Stability. At the end of 10 hours, all three lots of cassettes produced expected results showing that ARIES® Flu A/B and RSV Assay Cassettes are stable in ambient laboratory conditions for up to ten (10) hours after they have been removed from the storage pouch.

Controls:*Process Control*

Each ARIES® assay cassette contains a Sample Process Control (SPC), which is processed with the sample and analyzed during the amplification reaction. The SPC verifies sample lysis, nucleic acid extraction, and proper reagent, cassette, ARIES® System, and assay protocol performance. The SPC has a known melting temperature (T_m) range and Ct range. Each time an assay is run, the system measures the temperature and fluorescence intensity of the SPC control to ensure the thermal and optical subsystems have remained in calibration.

External Controls

External controls should be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. For example, reference influenza A, influenza B & RSV strains or well characterized influenza A, influenza B, and RSV clinical isolates may be used as positive controls. The ARIES® Flu A/B & RSV Assay Cassette Kit does not include external positive and negative controls.

d. Detection Limit:

Limit of Detection (LoD) was established for the ARIES® Flu A/B & RSV Assay using three influenza A, two influenza B, and two respiratory syncytial viral strains (1 RSV-A, and 1 RSV-B) diluted in a simulated nasal matrix (SNM) containing negative pooled human clinical matrix and Universal Transport media (UTM). The LoD for each viral strain was determined as the lowest concentration (TCID₅₀/mL) that had a positivity rate of $\geq 95\%$. Serial dilutions of each quantified viral strain in SNM were initially tested in a range finding study where the preliminary LoD TCID₅₀/mL concentrations were determined. The preliminary LoD concentrations were then confirmed by testing twenty (20) replicates of each strain. The final LoD concentrations for the seven viral strains are shown in Table 11-6.

Table 11-6: Limit of Detection of the ARIES® Flu A/B & RSV Assay

Assay Target	Strain	Concentration (TCID ₅₀ /mL or CEID ₅₀ /mL)	Positivity	95% Confidence Interval (Lower Limit / Upper Limit)
Influenza A	PR/8/34	1 x 10 ^{-0.34}	100%	83.2% - 100%
	Hong Kong/8/68	1 x 10 ^{2.40}	100%	83.2% - 100%
	Mexico/4108/2009 (H1N1)pdm09	1 x 10 ^{1.45}	95%	75.1% - 99.9%
Influenza B	Florida/04/06	1 x 10 ^{0.30}	100%	83.2% - 100%
	Malaysia/2506/04	1 x 10 ^{1.05}	100%	83.2% - 100%
RSV	A2 (VR-1540)	1 x 10 ^{-0.57}	95%	75.1% - 99.9%
	WV/14617/85 (VR-1400)	1 x 10 ^{0.90}	100%	83.2% - 100%

e. Analytical Reactivity (Inclusivity)

The analytical reactivity/inclusivity of the ARIES® Flu A/B and RSV Assay was evaluated against twenty-four influenza A strains, seven influenza B strains, and three respiratory syncytial virus (RSV) strains, which differ from those strains included in the Limit of Detection (LoD) study. Each strain was diluted in simulated nasal matrix (SNM) to a concentration near the LoD (1x10¹ TCID₅₀/mL or CEID₅₀/mL) and tested in triplicate; SNM consists of a 1:1 mixture of Universal Transport Medium (UTM) and 100% negative clinical specimen pool. For five strains, additional testing at 1x10² TCID₅₀/mL or CEID₅₀/mL was required to achieve 100% positivity. For two strains, additional testing at 1x10³ TCID₅₀/mL or CEID₅₀/mL was required to achieve 100% positivity. The study results are shown in Table 11-7.

Table 11-7: ARIES® Flu A/B & RSV Assay Analytical Reactivity/Inclusivity Results

Inclusivity Strains	100% Positivity Concentration	Result
Influenza A /Perth/16/2009 (H3N2)-like	1x10 ¹	Influenza A Detected
Influenza A/Brisbane/10/07 H3	1x10 ¹	Influenza A Detected
Influenza A/Brisbane/59/07 H1	1x10 ¹	Influenza A Detected
Influenza A/Port Chalmers/1/73 H3N2	1x10 ¹	Influenza A Detected
Influenza A/Solomon Island/03/06 H1	1x10 ¹	Influenza A Detected
Influenza A/Swine H1N1/Iowa/15/1930	1x10 ¹	Influenza A Detected
Influenza A/Taiwan/42/06 H1N1	1x10 ¹	Influenza A Detected
Influenza A/Wisconsin/67/05 H3	1x10 ¹	Influenza A Detected
Influenza A/California/7/2009-like (pH1N1)	1x10 ¹	Influenza A Detected

Influenza A/Hong Kong/33982/2009 H9N2 x PR8-	1x10 ¹	Influenza A Detected
Influenza A/Indiana/08/2011 (H3N2)v	1x10 ¹	Influenza A Detected
Influenza A/Texas/50/2012 H3N2	1x10 ¹	Influenza A Detected
Influenza A/WS/33 H1N1	1x10 ¹	Influenza A Detected
Influenza A/New Caledonia/20/99 H1N1	1x10 ²	Influenza A Detected
Influenza A/Swine H1N1/USA/1976/1931	1x10 ²	Influenza A Detected
Influenza A/California/07/2009 NYMC x-179A	1x10 ²	Influenza A Detected
Influenza A/Victoria/361/2011-like (H3N2)	1x10 ²	Influenza A Detected
Influenza A/Minnesota/11/2010 (H3N2)v	1x10 ³	Influenza A Detected
Influenza A/Ohio/02/2012 (H3N2)	1x10 ³	Influenza A Detected
A/Anhui/01/2005 (H5N1) ^a	1x10 ¹	Influenza A Detected
A/Anhui/1/2013 (H7N9) ^a	1x10 ¹	Influenza A Detected
A/Egypt/321/2007 (H5N1) ^a	1x10 ¹	Influenza A Detected
A/Shanghai/1/2013 (H7N9) ^a	1x10 ¹	Influenza A Detected
A/Vietnam/1194/2004 (H5N1) ^a	1x10 ¹	Influenza A Detected
Influenza B/Massachusetts/2/2012-like	1x10 ¹	Influenza B Detected
Influenza B/Wisconsin/1/2010-like	1x10 ¹	Influenza B Detected
Influenza B/Florida/02/2006 (Victoria)	1x10 ¹	Influenza B Detected
Influenza B/Lee/40	1x10 ¹	Influenza B Detected
Influenza B/Panama/45/90 (Yamagata)	1x10 ¹	Influenza B Detected
Influenza B/Brisbane/60/2008	1x10 ¹	Influenza B Detected
Influenza B/Florida/07/04 (Yamagata)	1x10 ²	Influenza B Detected
RSV A/Long	1x10 ¹	RSV Detected
RSV B/9320	1x10 ¹	RSV Detected
RSV B/Wash/18537/62	1x10 ¹	RSV Detected

^a BPL-inactivated viral culture fluid provided from IRR with no unit/titer information. Dilution factors based on prior preliminary LoD testing of these strains with the ARIES® Flu A/B & RSV assay to approximate limit of detection.

f. Analytical specificity:

Interfering Substances:

The potential inhibitory effect of non-microbial substances expected to be found in nasopharyngeal swab specimens was evaluated by the testing with the ARIES® FLU A/B & RSV Assay. Three (3) replicates each of influenza A, influenza B, RSV-A, and RSV-B were tested at concentrations near the assay LoD with a clinically relevant

concentration of each potentially interfering substance spiked into the contrived sample; additionally, negative Copan Universal Transport Medium (UTM) was spiked with the same concentration of each substance and tested for assay interference.

The results of the study demonstrate that all influenza A, influenza B, and RSV samples were 100% positive in the presence of a non-microbial substance at concentrations shown below; all negative samples containing only the non-microbial substance were 100% negative, with the exception of FluMist®.

Positive influenza results obtained in a patient who received FluMist® prior to sample collection may be due to detection of the live attenuated influenza vaccine virus and may mask a true positive result caused by an influenza infection; additionally, FluMist® may interfere with RSV detection due to high concentration of vaccine virus nucleic acid, causing a possible RSV false negative result. This statement has been added to the Analytical Performance section of the ARIES® FLU A/B & RSV Product Insert.

Table 11-8: Interfering Substances Tested

Interfering Substance	Test Concentration
Benzocaine	2.5% w/v
Budesonide	25 mg/mL
Dexamethasone	3 mg/mL
FluMist®	0.5% v/v
Flunisolide	55 mg/mL
Menthol	1.7 mg/mL
Mometasone	2.5 mg/mL
Phenylephrine	0.5% w/v
Afrin® (Oxymetazoline)	15% v/v
Tobramycin	4 µG/mL
Mupirocin	6.6 mg/mL
Beconase AQ® (Beclomethasone)	5% v/v
Flonase® (Fluticasone)	5% v/v
Zanamivir	3.3 mg/mL
Tamiflu®	1 µM
Triamcinolone	5.5 mg/mL
Sodium chloride	0.65% v/v
Human Whole Blood	2% v/v
Mucin Protein	60 µG/mL
ZICAM® (Galphimia glauca, Histaminum hydrochloricum, Luffa operculata, Sulfur)	5% v/v

Cross-Reactivity:

The analytical specificity for the ARIES® Flu A/B & RSV Assay was evaluated by testing the potential cross-reactivity of 32 microorganisms listed in Table 11-9. The microorganisms tested consisted of 14 viral and 18 bacterial strains representing common respiratory pathogens, or those potentially encountered in the human nasopharynx region. The potential cross reactive organisms were spiked into simulated nasal matrix (SNM) that was negative for influenza A, influenza B, and RSV and tested with the ARIES® Flu A/B & RSV Assay in triplicate. Bacterial organisms were tested at concentrations $\geq 10^6$ CFU/mL and viral organisms at $\geq 10^5$ TCID₅₀/mL, or the highest available concentration for both types of potential cross-reactive microorganism. All replicates of 31 microorganisms that were evaluated yielded negative results following initial testing. One replicate of three tested for Parainfluenza type 1 generated a late Ct indicating a weak influenza B positive result (Ct = 40) during the initial testing. An additional five replicates of the same Parainfluenza type 1 strain was tested with all 5 replicates yielding negative target results. Parainfluenza type 1 was considered to be non-reactive with the ARIES® Flu A/B & RSV Assay after the repeat testing.

Table 11-9: Microorganism Information

Microorganism		
Adenovirus type 1	<i>Haemophilus influenzae</i>	Parainfluenza type 1
Adenovirus 7a	<i>Mycoplasma pneumonia M129</i>	Parainfluenza type 2
<i>Bordetella pertussis (A639)</i>	<i>Mycobacterium tuberculosis</i>	Parainfluenza type 3
<i>Chlamydia pneumoniae</i>	<i>Lactobacillus plantarum (17-5)</i>	<i>Pseudomonas aeruginosa</i>
Coronavirus 229E	<i>Legionella longbeachae</i>	Rhinovirus type 1A
Coronavirus OC43	Measles	<i>Staphylococcus aureus (COL)</i>
<i>Corynebacterium diphtheriae</i>	Metapneumovirus	<i>Staphylococcus epidermidis</i>
Cytomegalovirus (CMV)	<i>Moraxella catarrhalis Ne 11</i>	<i>Streptococcus pneumoniae</i>
Enterovirus 71	Mumps	<i>Streptococcus pyogenes</i>
Epstein Barr virus	<i>Neisseria elongata</i>	<i>Streptococcus salivarius</i>
<i>Escherichia coli O157</i>	<i>Neisseria meningitidis</i>	

Microbial Interference:

Microbial interference for the ARIES® Flu A/B & RSV Assay was assessed with the 32 potential cross reactive microorganisms evaluated in the Cross-Reactivity Study, and identified in Table 11-9 above. Bacteria were tested at $\geq 10^6$ CFU/mL or the highest available concentration, and viruses were tested at $\geq 10^5$ TCID₅₀/mL or the highest available concentration. The potential interfering organisms were spiked into simulated nasal matrix (SNM) containing either representative strains of influenza A, influenza B, RSV A, or RSV B near the LoD concentration. All target strain + cross reacting organism samples were tested in triplicate (n=3) on the ARIES® System.

Influenza A was correctly detected in all replicates. Influenza B was correctly detected in the presence of 29 cross reacting organisms, with 3 cross reacting organisms (Parainfluenza type 2, Coronavirus OC43, *Corynebacterium diphtheriae*) requiring additional testing of 5 replicates. For all repeated cross reacting organism + influenza B combinations, all 5 replicates correctly detected influenza B. Parainfluenza type 2, Coronavirus OC43, and *Corynebacterium diphtheriae* are considered non-reactive since 7 of 8 tested replicates generated correct results (initial testing of 3 replicates resulted in 2 out of 3 positive, and the repeat testing of 5 replicates resulted in 5 out of 5 positive results, denoted as 7 of 8).

RSV A was correctly detected in the presence of 28 cross reacting organisms, with 4 cross reacting organisms (*Bordetella pertussis*, Cytomegalovirus, Parainfluenza type 2, Epstein Barr virus) requiring additional testing of 5 replicates. For all repeated cross reacting organism + RSV A combinations, all 5 replicates correctly detected RSV A. *Bordetella pertussis*, Cytomegalovirus, Parainfluenza type 2, and Epstein Barr virus are considered non-reactive since 7 of 8 tested replicates generated correct results. RSV B was correctly detected in the presence of 28 cross reacting organisms, with 4 cross reacting organisms (Parainfluenza type 2, Rhinovirus type 1A, Enterovirus 71, *Neisseria meningitidis*) requiring additional testing of 5 replicates. For all repeated cross reacting organism + RSV B combinations excluding Enterovirus 71, all 5 replicates correctly detected RSV A. Parainfluenza type 2, Rhinovirus type 2, and *Neisseria meningitidis* are considered non-reactive since 7 of 8 tested replicates generated correct results. RSV B had an overall positivity of 6/8 when tested in the presence of Enterovirus 71. Additional testing of RSV B + Enterovirus 71 produced a final positivity of 3/3 using freshly prepared target material. Based on the final test result of 100% positivity (3/3) for RSV B + Enterovirus 71, Enterovirus 71 is considered non-reactive.

Carry-Over/Cross-Contamination:

Carry-over and Cross Contamination for the ARIES® Flu A/B and RSV Assay were evaluated by testing thirty (30) high concentration influenza A positive samples in series alternating with thirty (30) influenza A negative samples (UTM). The high positive samples were run adjacent to negative samples across ten (10) consecutive runs on one ARIES® System. No carry-over or cross contamination was observed, and the overall percent agreement was 100% for positive and negative samples.

Competitive Interference/Co-infection:

A study was designed to evaluate the ability of the ARIES® Flu A/B and RSV assay to detect influenza A, influenza B, RSV A, and RSV B in the presence of a co-infection. Competitive interference can occur when one analyte is near the LoD and an additional analyte is present at high concentration. Analytes were tested at high (> 1x10⁵ TCID50/mL) and low concentrations (1.5X LoD) using 3 replicates in various combinations, excluding RSV A + RSV B due to the assay’s inability to distinguish between RSV subtypes. Low concentration analytes were detected for 3 of the 4 assay targets with 100% positivity in all high concentration combinations. RSV A in presence of high concentration influenza A required a 3X LoD dilution to achieve 100% positivity (Table 11-10).

Table 11-10: Co-infection Results Summary

Low Target Analyte	High Target Analyte	Condition	Percent
A/Hong Kong/8/68	B/Florida/04/06	Influenza A + Influenza B	100%
	RSV A2	Influenza A + RSV A	100%
	RSV B WV/14617/85	Influenza A + RSV B	100%
B/Florida/04/06	A/Hong Kong/8/68	Influenza B + Influenza A	100%
	RSV A2	Influenza B + RSV A	100%
	RSV B WV/14617/85	Influenza B + RSV B	100%
RSV A2	B/Florida/04/06	RSV A + Influenza B	100%
RSV A2 (3X LoD)	A/Hong Kong/8/68	RSV A + Influenza A	100%
RSV B WV/14617/85	A/Hong Kong/8/68	RSV B + Influenza A	100%
	B/Florida/04/06	RSV B + Influenza B	100%

g. Assay cut-off:

Not applicable.

2. Comparison Studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

A matrix equivalency study was performed to establish equivalency of a simulated matrix (50:50 dilution of pooled negative clinical specimens in Copan Universal Transport Media (UTM)) with a natural matrix (pooled negative clinical specimens)

when used in the ARIES® Flu A/B and RSV Assay. Study samples were prepared as a 4 point, 5-fold dilution series of representative Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) viral strain targets in both the simulated matrix and the pooled negative clinical matrix with the lowest dilution point targeting 3-5x the determined limit of detection. Each concentration level for each viral target strain was tested in 6 replicates for each matrix type for a total of 48 tests per viral strain. Additionally, 6 replicates of the simulated matrix and 6 replicates of the pooled negative clinical matrix were tested without the introduction of viral strain material. Equivalency was demonstrated based on the compared mean target Ct and SPC Ct values falling within 1 log (3.3 cycles) across the matrix types, and the accuracy of the clinical call in the simulated matrix compared with that of the natural matrix. The results of the study are shown in Tables 11-11 to 11-14. These results demonstrate the analytical performance of the ARIES® Flu A/B and RSV Assay is equivalent between the simulated matrix and the natural matrix.

Table 11-11: ARIES® Flu A/B and RSV Assay Matrix Equivalency Results for Flu A

Matrix type	Influenza A/Hong Kong/8/68 H3N2											
	Dilution 1			Dilution 2			Dilution 3			Dilution 4		
	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct
Simulated matrix	26.2 ± 0.7	100%		28.4 ± 0.4	100%		32.1 ± 0.8	100%		33.8 ± 1.3	100%	
Negative Clinical Specimen matrix	26.3 ± 1.1	100%	-0.1	29.4 ± 1.0	100%	-1.0	30.8 ± 0.4	100%	1.3	34.0 ± 1.0	83% ^a	-0.2

^a Positivity is within the 95% CI of expected results of 35.9% - 99.6%

Table 11-12: ARIES® Flu A/B and RSV Assay Matrix Equivalency Results for Flu B

Matrix type	Influenza B/Florida/04/06											
	Dilution 1			Dilution 2			Dilution 3			Dilution 4		
	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct

Simulated matrix	28.0 ± 0.5	100%	0.1	30.2 ± 0.7	100%	-1.2	33.0 ± 0.6	100%	0.2	35.4 ± 0.8	100%	1.0
Negative Clinical Specimen matrix	28.1 ± 0.3	100%		31.4 ± 0.7	100%		32.9 ± 0.8	100%		34.4 ± 0.9	100%	

Table 11-13: ARIES® Flu A/B and RSV Assay Matrix Equivalency Results for RSV

Matrix type	RSV A2											
	Dilution 1			Dilution 2			Dilution 3			Dilution 4		
	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct	Mean Ct ± SD (cycles)	Positivity	Delta Ct
Simulated matrix	30.9 ± 0.9	100%	0.2	32.5 ± 0.6	100%	-0.6	34.9 ± 0.7	100%	-0.7	35.3 ± 1.4	100%	-0.4
Negative Clinical Specimen matrix	30.8 ± 0.6	100%		33.1 ± 0.6	100%		35.6 ± 1.0	100%		35.6 ± 0.8	100%	

Table 11-14: ARIES® Flu A/B and RSV Assay Matrix Equivalency Results for SPC

Matrix type	SPC		
	Mean Ct ± SD (cycles)	Positivity	Delta Ct
Simulated matrix	27.6 ± 0.7	100%	0.0
Negative Clinical Specimen matrix	27.6 ± 0.8	100%	

c. Swab comparison:

A Nasopharyngeal Swab Equivalency study was performed to evaluate the reproducibility of the ARIES® Flu A/B & RSV Assay using three representative nasopharyngeal swab (NPS) types (flocked, rayon, and polyester) and two different swab sizes (minitip and regular). All swab types and swab sizes were evaluated with influenza A, influenza B, and RSV viral cultures prepared as a three point five-fold dilution series, transferred via select swab type and size to universal transport media (UTM), and tested with the ARIES® Flu A/B & RSV assay. In addition to the viral

cultures, a negative sample consisting of swab transferred UTM was also evaluated. Overall, all swab types and sizes performed as expected generating 100% positivity for all evaluated target concentrations and 100% negativity for negative replicates (Table 11-15).

Table 11-15: ARIES® Flu A/B & RSV Assay NPS Equivalency Results

Assay Target	Final Concentration (TCID ₅₀ /mL)	Swab Type	Regular Size Positivity	Minitip Size Positivity
A/Hong Kong/8/68 LoD = 1x10 ^{-2.40}	1x10 ^{4.5}	Flocked	100%	100%
	1x10 ^{3.8}		100%	100%
	1x10 ^{3.1}		100%	100%
	1x10 ^{4.5}	Polyester	100%	100%
	1x10 ^{3.8}		100%	100%
	1x10 ^{3.1}		100%	100%
	1x10 ^{4.5}	Rayon	100%	100%
	1x10 ^{3.8}		100%	100%
	1x10 ^{3.1}		100%	100%
B/Florida/04/06 LoD = 1x10 ^{-0.30}	1x10 ^{2.4}	Flocked	100%	100%
	1x10 ^{1.7}		100%	100%
	1x10 ^{1.0}		100%	100%
	1x10 ^{2.4}	Polyester	100%	100%
	1x10 ^{1.7}		100%	100%
	1x10 ^{1.0}		100%	100%
	1x10 ^{2.4}	Rayon	100%	100%
	1x10 ^{1.7}		100%	100%
	1x10 ^{1.0}		100%	100%
RSV A2 LoD = 1x10 ^{-0.57}	1x10 ^{1.5}	Flocked	100%	100%
	1x10 ^{0.8}		100%	100%
	1x10 ^{0.1}		100%	100%
	1x10 ^{1.5}	Polyester	100%	100%
	1x10 ^{0.8}		100%	100%
	1x10 ^{0.1}		100%	100%
	1x10 ^{1.5}	Rayon	100%	100%
	1x10 ^{0.8}		100%	100%
	1x10 ^{0.1}		100%	100%

3. Clinical Performance:

The clinical performance of the ARIES® Flu A/B & RSV Assay was evaluated using leftover, de-identified, nasopharyngeal swab (NPS) specimens prospectively collected from pediatric or adult patients suspected of having respiratory tract infection during the 2014/2015 and 2015/2016 flu seasons. In the first phase of the prospective study (2014/2015 flu season) specimens were collected at 3 clinical sites located in the United States and Canada from 18-January-2015 to 20-March-2015. Clinical specimens accrued during the second phase of the prospective study (2015/2016 flu season) were collected from 02-November-2015 to 29-February-2016 at 4 clinical sites located in the United States and Canada. The clinical specimen collection sites were chosen based on the

types of patients usually referred to them, and the prevalence of respiratory pathogens in order to ensure a broad coverage of respiratory organisms, patient ages, and geographical regions.

A total of 2504 nasopharyngeal swab specimens from subjects suspected of having respiratory tract infection were collected in the prospective study. Twenty-five (25) specimens were excluded based on inclusion/exclusion criteria violation or protocol deviation leaving a total of 2479 eligible unique specimens for subsequent data analysis. Of these, 1017 were collected during the 2014/2015 Flu season while the remaining 1462 specimens were enrolled during the 2015/2016 Flu season.

All 2479 eligible clinical specimens were tested by an FDA-cleared molecular comparator and the ARIES® Flu A/B & RSV Assay. The comparator assay was performed in accordance with manufacturer’s instructions at a centralized testing facility. Clinical runs and re-runs using ARIES® Flu A/B & RSV Assay were carried out by trained operators at the testing sites on clinical specimens that were either stored frozen at -65°C to -80°C (N=1316; 53.1%) or kept refrigerated at 2°C to 8°C (N=1163; 46.9%) prior to testing.

Out of the 2479 clinical specimens included in the prospective analysis, 2458 (2458/2479; 99.2%) generated valid results with ARIES® Flu A/B & RSV Assay during initial testing. There were 21 specimens (21/2479, 0.8%) that were re-tested with ARIES® Flu A/B & RSV Assay because they yielded initial invalid results. All 21 specimens in question generated valid ARIES results upon repeat testing.

ARIES® Flu A/B & RSV Assay Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for Influenza A, Influenza B and RSV are summarized in the tables below. Discordant specimens where the ARIES® Flu A/B & RSV Assay results were different from the comparator assay result were further assessed by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay. Results from discordant testing analysis were not included in the calculation of Positive Percent Agreement and Negative Percent Agreement for each target. These results are, however, included as footnotes in the performance evaluation tables for each analyte.

Table 11-16: ARIES® Flu A/B & RSV Assay Performance for Influenza A (N=2479)

ARIES® Flu A/B & RSV Assay	Reference			TOTAL
	Positive	Negative	No Call	
Positive	299	34 ²	1	334
Negative	13 ¹	2131	1	2145
No Call	0	0	0	0
TOTAL	312	2165	2	2479
		95% CI		

Positive Percent Agreement	95.8%	93.0% - 97.8%		
Negative Percent Agreement	98.4%	97.8% - 98.9%		

¹ Seven (7) ARIES® Flu A/B & RSV Assay negative specimens that were positive by the comparator method (i.e. False Negative) tested negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay.

² Four (4) ARIES® Flu A/B & RSV Assay positive specimens that were negative by the comparator method (i.e. False Positive) tested positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay.

Table 11-17: ARIES® Flu A/B & RSV Assay Performance for Influenza B (N=2479)

ARIES® Flu A/B & RSV Assay	Reference			TOTAL
	Positive	Negative	No Call	
Positive	45	14 ²	0	59
Negative	3 ¹	2417	0	2420
No Call	0	0	0	0
TOTAL	48	2431	0	2479
		95% CI		
Positive Percent Agreement	93.8%	82.8% - 98.7%		
Negative Percent Agreement	99.4%	99.0% - 99.7%		

¹ Two (2) ARIES® Flu A/B & RSV Assay negative specimens that were positive by the comparator method (i.e. False Negative) tested negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay.

² Three (3) ARIES® Flu A/B & RSV Assay positive specimens that were negative by the comparator method (i.e. False Positive) tested positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay.

Table 11-18: ARIES® Flu A/B & RSV Assay Performance for RSV (N=2479)

ARIES® Flu A/B & RSV Assay	Reference			TOTAL
	Positive	Negative	No Call	
Positive	270	36 ²	0	306
Negative	8 ¹	2165	0	2173
No Call	0	0	0	0
TOTAL	278	2201	0	2479
		95% CI		
Positive Percent Agreement	97.1%	94.4% - 98.7%		
Negative Percent Agreement	98.4%	97.7% - 98.9%		

¹ One (1) ARIES® Flu A/B & RSV Assay negative specimen that was positive by the comparator method (i.e. False Negative) tested negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay.

² Thirty-two (32) ARIES® Flu A/B & RSV Assay positive specimens that were negative by the comparator method (i.e. False Positive) tested positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Flu A/B & RSV Assay.

Due to the low prevalence of Influenza B observed in the prospective study, the sample set was supplemented with 40 banked (pre-selected) Influenza B positive specimens collected at a single clinical laboratory (collection site) in Canada. The presence of the expected target (Influenza B) in each of the pre-selected specimens was confirmed by the comparator assay. In order to minimize bias, the pre-selected positive specimens were tested along with 40 unique negative clinical specimens in a randomized, blinded fashion at 4 external testing sites. Of the 80 Influenza B positive and negative specimens included in this study, 78 (78/80; 97.5%) generated valid results with ARIES® Flu A/B & RSV Assay on the first attempt. Invalid results were generated for 2 specimens (2/80; 2.5%) tested. Both specimens in question yielded valid ARIES results upon repeat testing.

The results from pre-selected specimens were analyzed separately from those of the prospective data set. ARIES® Flu A/B & RSV Assay accurately detected all 40 Influenza B positive specimens tested (100%; 95% confidence interval, 91.2% - 100%).

Based on the data collected from the clinical study, Positive Percent Agreement acceptance criteria of 90% with a lower bound 95% confidence interval of at least 80% were achieved for Influenza A, Influenza B and RSV. Negative Percent Agreement acceptance criteria of 90% with a lower bound 95% confidence interval of at least 90% were also achieved for all targets probed by the ARIES® Flu A/B & RSV Assay.

The results generated from this clinical study demonstrate that the diagnostic accuracy of the ARIES® Flu A/B & RSV Assay is acceptable for the detection of Influenza A, Influenza B and RSV in nasopharyngeal swabs collected from patients suspected of respiratory tract infection (RTI).

4. Expected values:

ARIES® Flu A/B & RSV Assay positive results (expected values) after allowable re-runs for each individual target are summarized for each of the enrollment periods, per age groups and per site in Tables 11-19 to 11-22 below.

Table 11-19: Expected Values (As determined by ARIES® Flu A/B & RSV) – Summary by Age Groups for the ARIES® Flu A/B & RSV Prospective Clinical Study (January 2015 – March 2015)

Target	Overall (n=1017)	0-1 year (n=190)	>1-5 years (n=92)	>5-21 years (n=131)	>21-65 years (n=307)	>65 years (n=297)
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(Analyte)	No.	Expected Value										
Influenza A	170	16.7%	8	4.2%	13	14.1%	25	19.1%	53	17.3%	71	23.9%
Influenza B	31	3.0%	1	0.5%	3	3.3%	11	8.4%	10	3.3%	6	2.0%
RSV	104	10.2%	50	26.3%	14	15.2%	4	3.1%	15	4.9%	21	7.1%

Table 11-20: Expected Values (As determined by ARIES® Flu A/B & RSV) – Summary by Age Groups for the ARIES® Flu A/B & RSV Prospective Clinical Study (November 2015 – February 2016)

Target (Analyte)	Overall (n=1462)		0-1 year (n=244)		>1-5 years (n=131)		>5-21 years (n=114)		>21-65 years (n=538)		>65 years (n=435)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Influenza A	164	11.2%	9	3.7%	17	13.0%	15	13.2%	98	18.2%	25	5.7%
Influenza B	28	1.9%	3	1.2%	2	1.5%	9	7.9%	9	1.7%	5	1.1%
RSV	202	13.8%	83	34.0%	33	25.2%	10	8.8%	24	4.5%	52	12.0%

Table 11-21: Expected Values (As determined by ARIES® Flu A/B & RSV) – Summary by Site for the ARIES® Flu A/B & RSV Prospective Clinical Study (January 2015 – March 2015)

Target (Analyte)	Overall (n=1017)		Site 01 (n=238)		Site 02 (n=225)		Site 04 (n=554)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Influenza A	170	16.7%	42	17.6%	39	17.3%	89	16.1%
Influenza B	31	3.0%	2	0.8%	17	7.6%	12	2.2%
RSV	104	10.2%	12	5.0%	20	8.9%	72	13.0%

Table 11-22: Expected Values (As determined by ARIES® Flu A/B & RSV) – Summary by Site for the ARIES® Flu A/B & RSV Prospective Clinical Study (November 2015 – February 2016)

Target (Analyte)	Overall (n=1462)		Site 01 (n=573)		Site 02 (n=102)		Site 03 (n=387)		Site 04 (n=400)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value
Influenza A	164	11.2%	74	12.9%	12	11.8%	16	4.1%	62	15.5%
Influenza B	28	1.9%	8	1.4%	4	3.9%	3	0.8%	13	3.3%
RSV	202	13.8%	58	10.1%	12	11.8%	53	13.7%	79	19.8%

N. Proposed Labeling:

The labeling provided in the submission satisfies the requirements of 21 CFR 809.10.

O. Conclusion:

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