510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K161220

B. Purpose for Submission:

Clearance of New Device

C. Measurand:

Influenza A RNA: Matrix gene
Influenza B RNA: Matrix gene
Respiratory Syncytial Virus (RSV) RNA: Fusion gene of RSV A and RSV B

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:

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

OOI – Real time nucleic acid amplification system

OZE - influenza A and influenza B multiplex nucleic acid assay

4. Panel:

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 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.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   For use with the ARIES System or ARIES M1 System

   Note: The ARIES System and ARIES M1 System are collectively referred to as the ARIES Systems throughout this memo.

I. Device Description:

The ARIES Flu A/B & RSV Assay is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test system that consists of the ARIES Systems 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, and an assay-specific master mix designed to perform the designated assay on one sample. The ARIES Flu A/B & RSV Assay cassette is used to directly detect and differentiate influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) nucleic acid in NPS specimens from patients with signs and symptoms of respiratory tract infection. The assay detects the matrix protein genes of influenza A and influenza B viruses, the fusion gene of RSV, and a RNA Sample Processing Control.

The specimen is lysed and nucleic acid is extracted using the ARIES Systems. 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 Ct (threshold cycle) 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 the real-time PCR instrument. The Tm (melting temperature) 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 contains target-specific primer pairs labeled with distinct fluorophore labels. PCR amplification is performed and assay fluorescence is monitored on the ARIES Systems. 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 Tm that is indicated by an increase in fluorescence. 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.

Materials Provided

- ARIES Flu A/B & RSV Assay Cassette Kit – contains 24 assay cassettes which contain necessary reagents for sample extraction, nucleic acid purification, and amplification.

- ARIES Flu A/B & RSV Assay Protocol File Kit – contains the assay protocol file, package insert, and ARIES Quick Guide provided on a USB drive.

Materials Required But Not Provided

Reagents for sample collection:

- Nasopharyngeal swab (NPS) (flocked, polyester, or rayon swab)
- Universal Transport Medium (UTM)

Equipment:

- -65°C to -95°C freezer
- 2°C to 8°C refrigerator
- Luminex ARIES Systems (either an ARIES System or an ARIES M1 System) and accessories
  - ARIES magazines
  - Sample Prep Tray
- Vortex mixer
- Appropriately sized pipettor

Plasticware and Consumables:

- Nuclease-free aerosol-barrier pipette tips

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Simplexa™ Flu A/B & RSV Direct
   Simplexa™ Flu A/B & RSV Positive Control Pack

2. Predicate 510(k) number(s):
   K120413

3. Comparison with predicate:

Similarities		
Item	**Subject Device ARIES Flu A/B & RSV Assay (K161220)**	**Predicate Simplexa™ Flu A/B & RSV Direct (K120413)**
Regulation	866.3980	866.3980
Product Code	OCC, OOI, OZE	OCC, OOI
Device Classification	II	II
Intended Use	The ARIES Flu A/B & RSV Assay is a polymerase chain reaction (PCR) based qualitative *in vitro* diagnostic test for the	The Focus Diagnostics Simplexa Flu A/B & RSV Direct assay is intended for use on the 3M Integrated Cycler

	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.	instrument for the *in vitro* qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) RNA in nasopharyngeal swabs (NPS) from human patients with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors. This test is intended for use as an aid in the differential diagnosis of influenza A, influenza B, and RSV viral infections in humans and is not intended to detect influenza C. Negative results do not preclude influenza virus or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Performance characteristics for influenza A were established with clinical specimens collected during the 2010/2011 influenza season when 2009 H1N1 influenza and H3N2 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to the state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
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

Differences		
Item	**Subject Device ARIES Flu A/B & RSV Assay (K161220)**	**Predicate Simplexa Flu A/B & RSV Direct, (K120413)**
Extraction Method	Automated by the ARIES Systems	None
RSV Target	Fusion gene	M gene
Controls	Sample Processing Control (SPC)	RNA Internal Control, Positive Control provided separately
Instrument	ARIES System or ARIES M1 System	3M™ Integrated Cycler

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

1. FDA Guidance: Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay, issued on October 9, 2009
2. FDA Guidance: Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses, issued on July 15, 2011
3. FDA Guidance: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path, issued on May 1, 2007.
4. CLSI EP5-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
5. CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
6. CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition
7. CLSI EP15-A3: User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition
8. CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition
9. CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline - First Edition
10. BS EN ISO 14971:2012: Medical devices - Application of risk management to medical devices

L. Test Principle:

Primary sample (NPS specimen in universal transport medium) is added directly to the ARIES Flu A/B & RSV Assay cassette sample chamber. The cassette is then placed into an ARIES magazine which can hold up to six cassettes. The magazine is inserted into an ARIES instrument. A barcode on top of the ARIES Flu A/B & RSV Assay cassette is automatically scanned by the ARIES instrument, associating a preloaded ARIES Flu A/B & RSV Assay Protocol File with the cassette. The ARIES Flu A/B & RSV Assay Protocol File contains the necessary parameters to run the cassette, analyze data, and generate reports.

Once a run is started, the sample processing control (SPC) is automatically added to the sample chamber of the cassette to control for sample lysis, recovery of extracted nucleic acid,

detection of inhibitory substances, and confirmation of PCR reagent integrity. Sample and SPC lysis, as well as isolation and purification of nucleic acids, are automated within the ARIES Systems and the ARIES Flu A/B & RSV Assay cassette. Purified nucleic acids are automatically transferred to the cassette's PCR tube that contains the lyophilized Influenza A/B & RSV Master Mix for the PCR amplification step. The lyophilized Influenza A/B & RSV Master Mix contains four sets of primers: one primer set for the SPC target, and an independent primer set for each pathogen.

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 the ARIES Systems. 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 Tm of the amplicon.

Quality Control

ARIES Flu A/B & RSV Assay Cassette contains a sample processing control, which is processed with the sample and analyzed during the amplification reaction.

External controls should be used in accordance with local, state, federal accrediting organizations, as applicable. For example, reference influenza A, influenza B and RSV strains or well characterized influenza A, influenza B and RSV clinical isolates may be used as positive controls; universal transport medium may be used as a negative control.

Interpretation of Results

The ARIES analysis software determines results for the sample and the SPC based on the amplification cycle (Ct) value and the melting temperature (Tm) value provided in the Assay Protocol File. All assay outcomes are described below.

Interpretation of ARIES Flu A/B & RSV Sample Result

Example [a]	SPC		Influenza A		Influenza B		RSV		Call
	Tm Value	Ct Value	Tm Value	Ct Value	Tm Value	Ct Value	Tm Value	Ct Value	
1	+	N/A	+	+	+	>	+	>	Influenza A Positive, Influenza B Negative, RSV Negative
2	+	N/A	+	+	-	N/A	-	N/A	
3	+	N/A	+	>	+	+	+	>	Influenza A Negative, Influenza B Positive, RSV Negative
4	+	N/A	-	N/A	+	+	-	N/A	
5	+	N/A	+	>	+	>	+	+	Influenza A Negative, Influenza B Negative, RSV Positive
6	+	N/A	-	N/A	-	N/A	+	+	
7	+	N/A	+	+	+	+	+	>	Influenza A Positive, Influenza B Positive, RSV Negative [b]
8	+	N/A	+	+	+	+	-	N/A	
9	+	N/A	+	+	+	>	+	+	Influenza A Positive, Influenza B Negative, RSV Positive
10	+	N/A	+	+	-	N/A	+	+	
11	+	N/A	+	>	+	+	+	+	Influenza A Negative, Influenza B Positive, RSV Positive
12	+	N/A	-	N/A	+	+	+	+	
13	+	N/A	+	+	+	+	+	+	Influenza A Positive, Influenza B Positive, RSV Positive [b]
14	+	+	+	>	+	>	+	>	Influenza A Negative, Influenza B Negative, RSV Negative
15	+	+	-	N/A	-	N/A	-	N/A	

[a] The scenarios not captured above will be called "Invalid". In case of an "Invalid" result, re-test the sample with a new assay cassette. If the problem is unresolved, please contact Luminex Technical Support.

[b] Dual infections of Influenza A and Influenza B are rare. If the test result is "Influenza A Positive" and "Influenza B Positive", the assay should be repeated with the same patient specimen, or if possible, with a newly collected specimen.

Legend			
+	Indicates that a valid Ct value is present.	-	Indicates that a valid Ct value is not present.
>	Indicates that the Ct value obtained is above the Ct cutoff.	N/A	Not applicable. All possible outcomes will result in the same call.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   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 sites on five non-consecutive days.

   The reproducibility panel was prepared containing a moderate positive (10x LoD), low positive (3x LoD), and high negative (0.2x LoD) independently for one influenza A (Influenza A/Hong Kong/8/68), one influenza B (Influenza B/Florida/04/06), one RSV-A (RSV A2), and one RSV-B (RSV B WV/14617/85) virus. Virus strains were formulated in a simulated nasal matrix (SNM) consisting of 50:50 dilution of pooled negative clinical specimens in universal transport media. A true negative sample consisted of only SNM. The reproducibility panel was prepared, blinded, randomized, and shipped frozen to each of 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 the following two tables.

Site-to-Site Reproducibility Results [a,b]

		Site 1		Site 2		Site 3		Total Positivity	95% Confidence Interval
		Positivity		Positivity		Positivity			
Influenza A	High Negative	27/30	90.0%	30/30	100.0%	26/30	86.7%	83/90 (92.2%)	84.6%-96.8%
	Low Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90 (100.0%)	95.9%-100%
	Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90 (100.0%)	95.9%-100%
	Negative	1/30	3.3%	0/30	0.0%	0/30	0.0%	1/90 (1.1%)	1.1%-60%
Influenza B	High Negative	9/30	30.0%	7/30	23.3%	15/30	50.0%	31/90 (34.4%)	24.7%-45.2%
	Low Positive	29/30	96.7%	30/30	100.0%	30/30	100.0%	89/90 (98.9%)	94.0%-100.0%
	Moderate Positive	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90 (100.0%)	95.9%-100%
	Negative	0/30	0.0%	0/30	0.0%	0/30	0.0%	0/90 (0.0%)	0.0%-4.0%
RSV[c]	High Negative	39/60	65.0%	50/60	83.3%	43/60	71.7%	132/180 (73.3%)	66.2%-79.6%
	Low Positive	60/60	100.0%	60/60	100.0%	60/60	100.0%	180/180(100.0%)	95.9%-100%
	Moderate Positive	60/60	100.0%	60/60	100.0%	59/60	98.3%	179/180 (99.4%)	96.9%-100.0%
	Negative	0/30	0.0%	0/30	0.0%	0/30	0.0%	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 results 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.

Site-to-Site Reproducibility Ct and Tm Results

		Site 1				Site 2				Site 3				Overall Results			
		Avg. Ct	**Ct %CV**	**Avg. Tm**	**Tm %CV**	**Avg. Ct**	**Ct %CV**	**Avg. Tm**	**Tm %CV**	**Avg. Ct**	**Ct %CV**	**Avg. Tm**	**Tm %CV**	**Avg. Ct**	**Ct %CV**	**Avg. Tm**	**Tm %CV**
Influenza A	Moderate Positive	31.5	9.84%	83.3	0.09%	32.2	2.39%	83.4	0.07%	32.2	2.28%	83.4	0.12%	32.0	5.94%	83.4	0.10%
	Low Positive	34.2	2.59%	83.4	0.11%	34.1	3.00%	83.4	0.11%	34.3	2.95%	83.3	0.10%	34.2	2.83%	83.4	0.11%
	High Negative	38.4	1.74%	83.2	0.12%	38.2	2.25%	83.3	0.11%	38.2	2.34%	83.2	0.10%	38.3	2.12%	83.2	0.11%
	Negative [a]	29.7	3.95%	78.5	0.27%	28.3	4.07%	78.8	0.31%	29.7	3.66%	78.4	0.38%				
Influenza B	Moderate Positive	35.0	2.30%	79.4	0.11%	35.2	1.97%	79.4	0.10%	35.1	1.70%	79.5	0.10%	35.1	2.00%	79.4	0.11%
	Low Positive	36.7	2.56%	79.5	0.10%	36.7	2.71%	79.4	0.12%	36.6	2.51%	79.4	0.10%	36.7	2.57%	79.4	0.11%
	High Negative	37.7	2.33%	79.4	0.13%	37.3	2.63%	79.4	0.10%	38.0	2.15%	79.4	0.07%	37.8	2.34%	79.4	0.09%
	Negative [a]	29.7	3.95%	78.5	0.27%	28.3	4.07%	78.8	0.31%	29.7	3.66%	78.4	0.38%				
RSV [b]	Moderate Positive	34.3	4.51%	75.9	0.34%	34.1	3.12%	75.9	0.36%	34.3	3.63%	75.9	0.33%	34.2	3.79%	75.9	0.34%
	Low Positive	36.0	3.92%	75.9	0.33%	35.6	3.49%	75.9	0.37%	35.8	4.05%	75.9	0.35%	35.8	3.83%	75.9	0.35%
	High Negative	37.3	4.65%	75.9	0.40%	37.0	4.21%	75.9	0.35%	37.3	4.00%	75.9	0.36%	37.2	4.27%	75.9	0.36%
	Negative [a]	29.7	3.95%	78.5	0.27%	28.3	4.07%	78.8	0.31%	29.7	3.66%	78.4	0.38%				
Flu A/B & RSV	Negative [a]													29.2	4.45%	78.5	0.38%

[a] Ct and Tm values for the Influenza A/B & RSV Negative reflects RNA SPC values.
[b] 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.

Within-Laboratory Precision

Within-Laboratory precision of the ARIES Flu A/B & RSV Assay was evaluated using the same panel described in the reproducibility study above. The panel was tested by two operators performing testing across multiple 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. The results of the precision study are summarized in the table below.

Within-Laboratory Precision Results

Strain	Target Concentration	Positivity	95% Confidence Interval	Mean Ct ± SD	Mean Tm ± SD
Influenza A	Moderate Positive	100% (48/48)	93% - 100%	32.9 ± 0.9	86.4 ± 0.1
	Low Positive	100% (48/48)	93% - 100%	35.0 ± 1.0	83.4 ± 0.1
	High Negative	79% (38/48)	65% - 90%	38.9 ± 0.7	83.2 ± 0.1
Influenza B	Moderate Positive	100% (48/48)	93% - 100%	32.7 ± 0.7	79.5 ± 0.1
	Low Positive	100% (48/48)	93% - 100%	34.5 ± 0.9	79.5 ± 0.1
	High Negative	81% (39/48)[a]	67% - 91%	37.1 ± 1.2	79.4 ± 0.1
RSV A	Moderate Positive	100% (48/48)	93% - 100%	34.5 ± 1.3	75.6 ± 0.1
	Low Positive	96% (46/48)	86% - 99%	36.1 ± 1.3	75.6 ± 0.1
	High Negative	48% (23/48)	33% - 63%	38.3 ± 1.5	75.6 ± 0.1
RSV B	Moderate Positive	100% (48/48)	93% - 100%	32.0 ± 1.2	76.1 ± 0.1
	Low Positive	100% (48/48)	93% - 100%	33.5 ± 2.3	76.1 ± 0.1
	High Negative	90% (43/48)[a]	77% - 97%	36.6 ± 1.8	76.1 ± 0.1
Flu A/B & RSV	Negative	2.1% (2/96)[b]	0% - 7%	28.0 ± 1.5	78.6 ± 0.3

[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%.

Lot-to-Lot Reproducibility

Lot-to-Lot Reproducibility of the ARIES Flu A/B & RSV Assay was evaluated by one operator using one ARIES System to test three lots of ARIES Flu A/B & RSV

cassettes. The test sample panel is the same as one described in the site-to-site reproducibility study above. A minimum of nine replicates of each target concentration were run three times for each cassette lot. The test results showed that the reproducibility across three cassette lots met the acceptance criteria of ≥ 95% for Moderate Positive, Low Positive and Negative Influenza A, Influenza B, RSV A, and RSV B samples.

b. *Linearity/assay reportable range:*

Not applicable; qualitative assay.

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

Stability:

Specimen Stability (Fresh vs. Frozen)

The specimen stability was determined with contrived fresh and frozen specimens stored at either 2-8°C (fresh) or -65°C to -95°C (frozen) and tested with the ARIES Flu A/B & RSV Assay. This was assessed by testing 60 replicates per single pathogen target (influenza A/Hong Kong/8/68, influenza B/Florida/04/06, or RSV A2) prepared at three test concentrations (30 low positive (2-3xLoD), 15 moderate positive (10xLoD), and 15 high positive (100xLoD)) across different time points extending up to 12 months. In addition to the 60 replicates for each target organism, 6 replicates of simulated nasal matrix were tested at each time point. Also, to address the effect of repeated freeze/thaw cycles on the assay performance, all five time point sample sets were initially frozen for one hour, thawed, and re-frozen prior to testing frozen samples at each time point. Results to date show that specimens for the ARIES Flu A/B & RSV Assay are stable for up to 7 days when stored at 2-8°C and up to 3 months when stored at -65° to -95°C.

Kit Stability

The real-time stability study was performed to evaluate the shelf life of ARIES Flu A/B & RSV Assay cassettes. Stability was established by testing six replicates of positive specimens and six replicates of negative specimens on three different lots of ARIES Flu A/B & RSV Assay cassettes stored at two different temperatures: 4°C (2–8°C) and room temperature (15–30°C) at 10 different time points extending up to 19 months. Results to date show that ARIES Flu A/B & RSV Assay cassettes can be stable for up to 5 months when stored at either 4°C (2–8°C) or room temperature (15–30°C).

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 hours. Testing was performed by one operator across three lots of cassettes on four ARIES Systems. Results show that ARIES Flu A/B and RSV Assay Cassettes are stable for up to 10 hours at room temperature after removal from the cassette pouch.

Controls:

Process Control

The sample processing control is present inside each test cassette to control sample lysis, nucleic acid extraction, and proper reagent, cassette, ARIES System, and assay protocol performance. The SPC has a known Tm 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 used in accordance with local, state, federal accrediting organizations, as applicable. For example, reference influenza A, influenza B, and RSV strains or well characterized influenza A, influenza B, and RSV clinical isolates may be used as positive controls; universal transport medium may be used as a negative control.

d. Detection limit:

The 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 virus strains diluted in a simulated nasal matrix. Preliminary LoD concentrations were determined by performing a six-point, five-fold dilution series of each virus strain tested. At least six replicates of each of the dilutions were tested to determine a preliminary LoD using one lot of ARIES Flu A/B & RSV cassettes. The LoD was defined as the lowest concentration that had a positivity rate of ≥ 95%.

The LoD concentrations determined in the preliminary study were confirmed by testing 20 replicates of the same virus strain diluted to the preliminary LoD concentrations. The final confirmed LoDs for each of the seven virus strains are presented in the table below.

Limit of Detection of the ARIES Flu A/B & RSV Assay

Assay Target	Strain	Confirmed LoD ($TCID_{50}$/ml or $CEID_{50}$/ml)	Positivity	95% Confidence Limit	Mean Ct ± SD
Influenza A	PR/8/34	$1X10^{-0.34}$	20/20 (100%)	83.2%-100%	37.9 ± 0.84
	Hong Kong/8/68	$1X10^{2.4}$	20/20 (100%)	83.2%-100%	36.4 ± 1.09
	Mexico/4108/2009 (H1N1)pdm09	$1X10^{1.45}$	19/20 (95%)	75.1%-99.9%	38.0 ± 0.92
Influenza B	Florida/04/06 (Yamagata)	$1X10^{0.3}$	20/20 (100%)	83.2%-100%	37.2 ± 1.33
	Malaysia/2506/04 (Victoria)	$1X10^{1.05}$	20/20 (100%)	83.2%-100%	36.4 ± 1.14
RSV	A2	$1X10^{-0.57}$	19/20 (95%)	75.1%-99.9%	37.0 ± 1.39
	WV/14617/85	$1X10^{0.9}$	20/20 (100%)	83.2%-100%	36.8 ± 1.43

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 LoD study. Each strain was diluted in the simulated nasal matrix to a concentration near the LoD ($1x10^1$ $TCID_{50}$/ml or $CEID_{50}$/ml) and tested in triplicate. For five strains, additional testing at $1x10^2$ $TCID_{50}$/ml or $CEID_{50}$/ml was required to achieve 100% positivity. For two strains, additional testing at $1x10^3$ $TCID_{50}$/ml or $CEID_{50}$/ml was required to achieve 100% positivity. The study results are shown in the table below.

Analytical Reactivity (Inclusivity) Results

Inclusivity Strains	100% Positivity Concentration	Result	Mean Ct ± SD	Mean Tm ± SD
Influenza A /Perth/16/2009 (H3N2)-like	$1x10^1$	Influenza A Detected	34.4 ± 0.4	84.4 ± 0.1
Influenza A/Brisbane/10/07 H3	$1x10^1$	Influenza A Detected	36.6 ± 0.1	83.3 ± 0.2
Influenza A/Brisbane/59/07 H1	$1x10^1$	Influenza A Detected	31.0 ± 0.8	83.5 ± 0.0
Influenza A/Port Chalmers/1/73 H3N2	$1x10^1$	Influenza A Detected	33.8 ± 0.5	83.4 ± 0.1
Influenza A/Solomon Island/03/06 H1	$1x10^1$	Influenza A Detected	35.1 ± 0.3	83.3 ± 0.1

Influenza A/Swine H1N1/Iowa/15/1930	$1x10^1$	Influenza A Detected	33.3 ± 0.9	83.1 ± 0.1
Influenza A/Taiwan/42/06 H1N1	$1x10^1$	Influenza A Detected	37.7 ± 0.9	83.3 ± 0.1
Influenza A/Wisconsin/67/05 H3	$1x10^1$	Influenza A Detected	34.0 ± 0.6	84.4 ± 0.1
Influenza A/California/7/2009-like (pH1N1)	$1x10^1$	Influenza A Detected	33.0 ± 0.5	83.0 ± 0.1
Influenza A/Hong Kong/33982/2009 H9N2 x PR8-IDCDC-RG26	$1x10^1$	Influenza A Detected	38.5 ± 0.8	83.2 ± 0.3
Influenza A/Indiana/08/2011 (H3N2)v	$1x10^1$	Influenza A Detected	35.2 ± 1.0	83.0 ± 0.0
Influenza A/Texas/50/2012 H3N2	$1x10^1$	Influenza A Detected	33.6 ± 0.7	83.5 ± 0.1
Influenza A/WS/33 H1N1	$1x10^1$	Influenza A Detected	35.3 ± 1.6	83.6 ± 0.0
Influenza A/New Caledonia/20/99 H1N1	$1x10^2$	Influenza A Detected	34.4 ± 1.0	83.4 ± 0.1
Influenza A/Swine H1N1/USA/1976/1931	$1x10^2$	Influenza A Detected	36.2 ± 2.7	83.0 ± 0.1
Influenza A/California/07/2009 NYMC x-179A	$1x10^2$	Influenza A Detected	36.7 ± 1.1	83.0 ± 0.1
Influenza A/Victoria/361/2011-like (H3N2)	$1x10^2$	Influenza A Detected	34.4 ± 0.4	83.5 ± 0.2
Influenza A/Minnesota/11/2010 (H3N2)v	$1x10^3$	Influenza A Detected	37.2 ± 0.6	83.7 ± 0.1
Influenza A/Ohio/02/2012 (H3N2)	$1x10^3$	Influenza A Detected	37.5 ± 1.1	84.2 ± 0.0
A/Anhui/01/2005 (H5N1)[a]	$1x10^1$	Influenza A Detected	27.6 ± 0.3	83.4 ± 0.1
A/Anhui/1/2013 (H7N9)[a]	$1x10^1$	Influenza A Detected	36.9 ± 0.9	83.8 ± 0.2
A/Egypt/321/2007 (H5N1)[a]	$1x10^1$	Influenza A Detected	35.0 ± 0.3	83.5 ± 0.1
A/Shanghai/1/2013 (H7N9)[a]	$1x10^1$	Influenza A Detected	37.4 ± 0.9	83.6 ± 0.3
A/Vietnam/1194/2004 (H5N1)[a]	$1x10^1$	Influenza A Detected	29.7 ± 0.4	83.3 ± 0.1

Influenza B/Massachusetts/2/2012-like	$1x10^1$	Influenza B Detected	35.1 ± 0.1	79.9 ± 0.1
Influenza B/Wisconsin/1/2010-like	$1x10^1$	Influenza B Detected	31.5 ± 1.2	79.9 ± 0.1
Influenza B/Florida/02/2006 (Victoria)	$1x10^1$	Influenza B Detected	37.4 ± 1.5	80.2 ± 0.1
Influenza B/Lee/40	$1x10^1$	Influenza B Detected	36.8 ± 1.1	78.3 ± 0.1
Influenza B/Panama/45/90 (Yamagata)	$1x10^1$	Influenza B Detected	34.3 ± 1.0	80.3 ± 0.1
Influenza B/Brisbane/60/2008	$1x10^1$	Influenza B Detected	33.2 ± 0.3	80.3 ± 0.1
Influenza B/Florida/07/04 (Yamagata)	$1x10^2$	Influenza B Detected	36.8 ± 1.2	79.5± 0.1
RSV A/Long	$1x10^1$	RSV Detected	34.4 ± 1.0	75.6 ± 0.1
RSV B/9320	$1x10^1$	RSV Detected	35.7 ± 0.3	75.7 ± 0.1
RSV B/Wash/18537/62	$1x10^1$	RSV Detected	34.8 ± 1.4	75.6 ± 0.1

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

All 34 influenza and RSV strains were correctly detected by the ARIES Flu A/B and RSV Assay at the indicated concentrations above.

f. Analytical Specificity:

Cross-Reactivity

This study was designed to determine potential cross-reactivity of the assay with 32 microorganisms listed in the table below.

Microorganisms Tested

#	Organism Name	Test Concentration	Units
1	*Chlamydia pneumoniaea*	$1.00 \times 10^{3.9}$	cfu/ml
2	*Haemophilus influenzae*	$1.00 \times 10^{6.1}$	cfu/ml
3	*Lactobacillus plantarum (17-5)*	$1.00 \times 10^{6.0}$	cfu/ml
4	Parainfluenza type 2	$1.00 \times 10^{4.4}$	$TCID_{50}$/ml
5	*Bordetella pertussis (A639)*	$1.00 \times 10^{6.1}$	cfu/ml

6	*Legionella longbeachae*	$1.00 \times 10^{6.0}$	cfu/ml
7	Parainfluenza type 3	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
8	Adenovirus type 1	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
9	*Pseudomonas aeruginosa*	$1.00 \times 10^{6.1}$	cfu/ml
10	*Moraxella catarrhalis Ne 11*	$1.00 \times 10^{5.7}$	cfu/ml
11	Measles	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
12	Cytomegalovirus (CMV)	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
13	Metapneumovirus	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
14	Rhinovirus type 1A	$1.00 \times 10^{4.8}$	$TCID_{50}$/ml
15	Coronavirus 229E	$1.00 \times 10^{4.9}$	$TCID_{50}$/ml
16	Epstein Barr virus	$1.00 \times 10^{5.0}$	copies/ml[a]
17	Enterovirus 71	$1.00 \times 10^{4.3}$	$TCID_{50}$/ml
18	Coronavirus OC43	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
19	Mumps	$1.00 \times 10^{4.7}$	$TCID_{50}$/ml
20	*Corynebacterium diphtheriae*	$1.00 \times 10^{6.0}$	cfu/ml
21	*Mycobacterium tuberculosis*	$1.00 \times 10^{5.8}$	cfu/ml
22	*Streptococcus pneumoniae*	$1.00 \times 10^{6.0}$	cfu/ml
23	*Streptococcus salivarius*	$1.00 \times 10^{6.0}$	cfu/ml
24	*Neisseria elongata*	$1.00 \times 10^{6.0}$	cfu/ml
25	*Neisseria meningitidis*	$1.00 \times 10^{6.0}$	cfu/ml
26	Parainfluenza type 1	$1.00 \times 10^{4.3}$	$TCID_{50}$/ml
27	Adenovirus 7a	$1.00 \times 10^{5.0}$	$TCID_{50}$/ml
28	*Staphylococcus epidermidis*	$1.00 \times 10^{6.0}$	cfu/ml
29	*Staphylococcus aureus (COL)*	$1.00 \times 10^{6.0}$	cfu/ml
30	*Escherichia coli O157*	$1.00 \times 10^{6.0}$	cfu/ml
31	*Mycoplasma pneumonia M129*	$1.00 \times 10^{6.0}$	ccu/ml[b]
32	*Streptococcus pyogenes*	$1.00 \times 10^{6.0}$	cfu/ml

[a] Copies/ml determined by quantitative PCR assay.
[b] Color Changing Unit (CCU) is calculated according to a modified Reed-Muench method based on the dilutions which produced a color change in the broth.

The microorganisms tested consisted of 14 viral and 18 bacterial strains representing common respiratory pathogens, or those potentially encountered in the human nasopharynx region. All organisms were diluted in the simulated nasal matrix that was negative for influenza A, influenza B, and RSV and tested by the ARIES Flu A/B & RSV Assay in triplicate. The bacterial strains were tested at concentrations $\geq 10^6$ cfu/ml and virus strains at $\geq 10^5$ $TCID_{50}$/ml, or the highest available concentration. Under the conditions of this study, thirty-one microorganisms were correctly reported as "influenza A negative; influenza B negative; RSV negative" by the ARIES Flu A/B & RSV Assay. For Parainfluenza type 1 strain, one of the three replicates

initially was reported as a weak influenza B positive (Ct=40). Additional five replicates of the same Parainfluenza type 1 strain were tested with all five replicates yielding negative target results.

Microbial Interference

Microbial interference for the ARIES Flu A/B & RSV Assay was assessed with the 32 potential cross reactive microorganisms. These bacterial and viral strains were tested at the same concentrations as described in the cross-reactivity study above. The potential interfering organisms were spiked into simulated nasal matrix containing either one strain of influenza A/Hong Kong/8/68, influenza B/Florida/04/06, RSV A2, or RSV B WV/14617/85 at a concentration of 3X LoD. All target strains added to cross reacting organism samples were tested in triplicate on the ARIES System. The results are summarized below.

Overall Results for Cross-Reacting Organisms (CRO)

CRO	Target				
	Flu A	Flu B	RSV A	RSV B	SNM
	Positivity	Positivity	Positivity	Positivity	Positivity
Chlamydia pneumoniae	3/3	3/3	3/3	3/3	0/3
Haemophilus influenzae	3/3	3/3	3/3	3/3	0/3
Lactobacillus plantarum (17-5)	3/3	3/3	3/3	3/3	0/3
Parainfluenza type 2	3/3	7/8[c]	7/8[c]	7/8[c]	0/3
Bordetella pertussis (A639)	3/3	3/3	7/8[c]	3/3	0/3
Legionella longbeachae	3/3	3/3	3/3	3/3	0/3
Parainfluenza type 3	3/3	3/3	3/3	3/3	0/3
Adenovirus type 1	3/3	3/3	3/3	3/3	0/3
Pseudomonas aeruginosa	3/3	3/3	3/3	3/3	0/3
Moraxella catarrhalis Ne 11	3/3	3/3	3/3	3/3	0/3
Measles	3/3	3/3	8/8[a]	3/3	0/3
Cytomegalovirus (CMV)	3/3	3/3	7/8[c]	3/3	0/3
Metapneumovirus	3/3	3/3	3/3	3/3	0/3
Rhinovirus type 1A	3/3	3/3	3/3	7/8[c]	0/3
Coronavirus 229E	3/3	3/3	3/3	3/3	0/3
Epstein Barr virus	3/3	3/3	7/8[c]	3/3	0/3
Enterovirus 71	3/3	3/3	3/3	3/3[b]	0/3
Coronavirus OC43	3/3	7/8[c]	3/3	3/3	0/3
Mumps	3/3	3/3	3/3	3/3	0/3
Corynebacterium diphtheriae	3/3	7/8[c]	3/3	3/3	0/3
Mycobacterium tuberculosis	3/3	3/3	3/3	3/3	0/3
Streptococcus pneumoniae	3/3	3/3	3/3	3/3	0/3

Streptococcus salivarius	3/3	3/3	3/3	3/3	0/3
Neisseria elongata	3/3	3/3	3/3	3/3	0/3
Neisseria meningitidis	3/3	3/3	3/3	7/8[c]	0/3
Parainfluenza type 1	3/3	3/3	3/3	3/3	1/8
Adenovirus 7a	3/3	3/3	3/3	3/3	0/3
Staphylococcus epidermidis	3/3	3/3	3/3	3/3	0/3
Staphylococcus aureus (COL)	3/3	3/3	3/3	3/3	0/3
Escherichia coli O157	3/3	3/3	3/3	3/3	0/3
Mycoplasma pneumonia M129	3/3	3/3	3/3	3/3	0/3
Streptococcus pyogenes	3/3	3/3	3/3	3/3	0/3

[a] Measles + RSV A was tested with eight replicates total due to operator errors.
[b] Enterovirus 71 + RSV B positivity (3/3) represents the re-testing of freshly prepared target material.
[c] Initial testing of three replicates resulted in two out of three positive results; subsequent testing with five replicates resulted in five out of five, or a total of seven out of eight positive results.

Under the conditions of this study, the results demonstrate that the ARIES Flu A/B and RSV assay does not cross-react with any of the 32 common respiratory microorganisms tested.

Competitive Interference/Co-infection

The 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 (> $1x10^5$ $TCID_{50}$/ml) and low concentrations (1.5X LoD) using three replicates in various combinations, excluding RSV A + RSV B due to the assay's inability to distinguish between RSV subtypes. The results for each analyte in combination with another competitive analyte are shown in the table below.

Co-infection Results Summary

Low Target Analyte	High Target Analyte	Condition	Percent	Low Target Mean Ct ± SD	High Target Mean Ct ± SD
A/Hong Kong/8/68	B/Florida/04/06	Influenza A + Influenza B	100%	37.7 ± 1.3	23.9 ± 0.8
	RSV A2	Influenza A + RSV A	100%	38.7 ± 0.5	22.5 ± 0.6
	RSV B WV/14617/85	Influenza A + RSV B	100%	38.1 ± 0.4	24.9 ± 0.4
B/Florida/04/06	A/Hong Kong/8/68	Influenza B + Influenza A	100%	37.5 ± 1.0	26.0 ± 0.3
	RSV A2	Influenza B + RSV A	100%	39.0 ± 0.6	22.3 ± 0.5
	RSV B WV/14617/85	Influenza B + RSV B	100%	38.4 ± 0.3	25.6 ± 0.5
RSV A2	A/Hong Kong/8/68	RSV A + Influenza A [a]	56%	36.7± 2.3	26.4 ± 1.1
	B/Florida/04/06	RSV A + Influenza B	100%	39.8 ± 0.2	22.9 ± 0.8
RSV A2 (3X LoD)[a]	A/Hong Kong/8/68	RSV A + Influenza A [a]	100%	38.4 ± 0.6	27.2 ± 1.3

RSV B WV/14617/85	A/Hong Kong/8/68	RSV B + Influenza A	100%	37.4 ± 1.4	24.5 ± 0.2
	B/Florida/04/06	RSV B + Influenza B	100%	39.9 ± 0.2	23.5 ± 0.2

[a] High concentration of influenza A had inhibitory effect on RSV A2 at a concentration lower than 3x LoD.

g. Interfering substances:

The potential inhibitory effect of non-microbial substances expected to be found in nasopharyngeal swab specimens was evaluated. Three replicates of each of influenza A/Hong Kong/8/68, influenza B/Florida/04/06, RSV A2, and RSV B WV/14617/85 strains were tested with the ARIES Flu A/B & RSV Assay at concentrations of 3X LoD with a clinically relevant concentration of each potentially interfering substance spiked into the contrived sample; additionally, universal transport medium was spiked with the same concentration of each substance and tested for assay interference. The evaluated substances are listed in the table below with tested concentrations shown.

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

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; all negative sample results 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 is addressed in the Analytical Performance section of the ARIES FLU A/B & RSV Product Insert and an appropriate limitation is included in the labeling.

h. Carry-Over/Cross-Contamination:

Carry-over and Cross-Contamination for the ARIES Flu A/B and RSV Assay were evaluated by testing 30 high concentration influenza A positive samples (A/Victoria/361/2011, 1x $10^{5.02}$ $TCID_{50}$/ml) in series alternating with 30 influenza A negative samples (i.e., universal transport medium). The high positive samples were run adjacent to negative samples across 10 consecutive runs on one ARIES System. Under the conditions of this study, no evidence of carry-over or cross contamination was observed in the ARIES System.

i. Assay cut-off:

For the ARIES Flu A/B and RSV Assay, each target (influenza A, Influenza B, RSV and sample processing control) has its own Ct cut-off value, Tm window, and Tm Peak Threshold, all of which are used to determine the assay result for the detection target as positive or negative. These assay parameters were determined by analysis of pre-clinical data derived from internal verification studies using the comparator assay (an FDA-cleared multiplex molecular test for respiratory pathogens). The parameter values are outlined below, and are hard-coded into the ARIES Flu A/B & RSV Assay Protocol File.

Assay Protocol File Parameters

Target	Tm(°C)	Ct (cycles)
Influenza A	81.4 - 85.6	≤ 40.0
Influenza B	77.6 - 82.2	≤ 40.5
RSV	74.4 -77.2	≤ 41.0
SPC	75.8 - 81.4	≤ 37.0

These assay protocol file parameter values were utilized in the determination of assay performance in the multi-site clinical trial conducted for the ARIES Flu A/B and RSV Assay.

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 nasal matrix consisting of 50:50 dilution of pooled negative clinical specimens in Copan universal transport medium versus a natural matrix (pooled negative clinical specimens) when used in the ARIES Flu A/B and RSV Assay. Study samples were prepared as a four-point, five-fold dilution series of representative Influenza A, Influenza B, and Respiratory Syncytial Virus strains in both the simulated nasal matrix and the pooled negative clinical matrix with the lowest dilution point targeting 3-5x LoD. Each concentration level for each viral target strain was tested in six replicates for each matrix type for a total of 48 tests per virus strain. Additionally, six replicates of the simulated nasal matrix and six replicates of the pooled negative clinical matrix were tested without the introduction of virus strain materials. The results of the study are shown in the tables below.

Matrix Equivalency Results for Influenza A

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

[a] Positivity is within the 95% CI of expected results of 35.9%-99.6%.

Matrix Equivalency Results for Influenza B

Matrix type	Influenza B/Florida/04/06											
	Dilution 1			Dilution 2			Dilution 3			Dilution 4		
	Mean Ct ± SD	Positivity	Delta Ct	Mean Ct ± SD	Positivity	Delta Ct	Mean Ct ± SD	Positivity	Delta Ct	Mean Ct ± SD	Positivity	Delta Ct
Simulated Nasal 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%	

Matrix Equivalency Results for RSV

Matrix type	RSV A2											
	Dilution 1			Dilution 2			Dilution 3			Dilution 4		
	Mean Ct ± SD	Positivity	Delta Ct	Mean Ct ± SD	Positivity	Delta Ct	Mean Ct ± SD	Positivity	Delta Ct	Mean Ct ± SD	Positivity	Delta Ct
Simulated Nasal 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%	

Matrix Equivalency Results for SPC

Matrix type	SPC		
	Mean Ct ± SD	Positivity	Delta Ct
Simulated Nasal Matrix	27.6 ± 0.7	100%	0.0
Negative Clinical Specimen Matrix	27.6 ± 0.8	100%	

Under the conditions of this study, there was no statistically significant effect in the performance of the ARIES Flu A/B & RSV assay when compared between the simulated nasal matrix and the natural matrix.

c. *Fresh versus frozen equivalency:*

Please refer to the "Specimen Stability (Fresh vs. Frozen)" of this document.

d. *Swab comparison:*

The 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 (mini-tip and regular). All swab types and swab sizes were evaluated with influenza A (A/Hong Kong/8/68), influenza B (B/Florida/04/06), and RSV (RSV A2) viral cultures prepared as a three point, five-fold dilution series, transferred via select swab type and size to Copan universal transport medium, and tested with the ARIES Flu A/B & RSV assay. In addition to the viral cultures, a negative sample consisting of 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.

3. Clinical studies:

The clinical performance of the ARIES Flu A/B & RSV Assay was evaluated using nasopharyngeal swab specimens prospectively collected at four geographically diverse locations representing intended use sites during the course of two influenza seasons (2014-2015 and 2015-2016). For the 2014-2015 season, two U.S. sites and one Canadian site participated in this study. For the 2015-2016 season, clinical specimens were collected and tested at a total of four sites including one additional U.S. site.

A total of 2,504 subject specimens were collected in the prospective study. Of these, twenty-five specimens were excluded in the study (12 specimens were not nasopharyngeal swabs (e.g. BALs, nasal aspirates, throat swabs), seven specimens generated No Call results using the reference method upon re-test and six specimens were excluded based on the violation of inclusion/exclusion criteria or protocol deviation). The demographic information of the study participants who provided the 2,479 eligible specimens is presented below.

Demographic Information for the Combined Prospective Dataset (N=2,479)

GENDER	Site 1	Site 2	Site 3	Site 4	All Sites
Male	375 (46.2%)	134 (41.0%)	200 (51.7%)	459 (48.1%)	1,168 (47.1%)
Female	436 (53.8%)	193 (59.0%)	187 (48.3%)	495 (51.9%)	1,311 (52.9%)
Total	811	327	387	954	2,479
AGE (years)					
0-1	37 (4.6%)	32 (9.8%)	102 (26.4%)	263 (27.6%)	434 (17.5%)
>1 – 5	29 (3.6%)	29 (8.9%)	62 (16.0%)	103 (10.8%)	223 (9.0%)
>5 – 21	29 (3.6%)	49 (15.0%)	64 (16.5%)	103 (10.8%)	245 (9.9%)
>21 – 65	316 (39.0%)	131 (40.1%)	124 (32.0%)	274 (28.7%)	845 (34.1%)
>65	400 (49.3%)	86 (26.3%)	35 (9.0%)	211 (22.1%)	732 (29.5%)
Total	811	327	387	954	2,479

Of these 2,479 eligible specimens, 1,017 were collected during the 2014-2015 Flu season while the remaining 1,462 specimens were collected during the 2015-2016 Flu season. Out of the 2,479 clinical specimens, 2,458 (2458/2479; 99.2%) generated valid results with ARIES Flu A/B & RSV Assay during initial testing. The overall invalid rate for the first-attempt testing was 0.8% (21/2479).

The performance of the ARIES Flu A/B & RSV Assay was compared to an FDA-cleared multiplex molecular test for respiratory pathogens. Results are shown in three tables below. Discordant samples were further analyzed by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from the ARIES Flu A/B & RSV Assay and subsequent results are documented in the footnotes.

Influenza A Clinical Performance Summary

ARIES Flu A/B & RSV Assay	Reference			
	Positive	Negative	No Call	TOTAL
Positive	299	34[b]	1	334
Negative	13[a]	2,131	1	2,145
No Call	0	0	0	0
Total	312	2,165	2	2,479
		95% CI		
Positive Percent Agreement	95.8%	93.0% - 97.8%		
Negative Percent Agreement	98.4%	97.8% - 98.9%		

[a] Seven ARIES Flu A/B & RSV Assay negative specimens that were positive by the reference method (i.e. False Negative) tested negative by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from the ARIES Flu A/B & RSV Assay.
[b] Four ARIES Flu A/B & RSV Assay positive specimens that were negative by the reference method (i.e. False Positive) tested positive by bi-directional sequencing using analytically validated primers that targeted genomic regions distinct from the ARIES Flu A/B & RSV Assay.

Influenza B Clinical Performance Summary

ARIES Flu A/B & RSV Assay	Reference			
	Positive	Negative	No Call	TOTAL
Positive	45	14[b]	0	59
Negative	3[a]	2,417	0	2,420
No Call	0	0	0	0
Total	48	2,431	0	2,479
		95% CI		
Positive Percent Agreement	93.8%	82.8% - 98.7%		
Negative Percent Agreement	99.4%	99.0% - 99.7%		

[a] Two ARIES Flu A/B & RSV Assay negative specimens that were positive by the reference 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.
[b] Three ARIES Flu A/B & RSV Assay positive specimens that were negative by the reference 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.

RSV Clinical Performance Summary

ARIES Flu A/B & RSV Assay	Reference			
	Positive	Negative	No Call	TOTAL
Positive	270	36[b]	0	306
Negative	8[a]	2,165	0	2,173
No Call	0	0	0	0
Total	278	2,201	0	2,479
		95% CI		
Positive Percent Agreement	97.1%	94.4% - 98.7%		
Negative Percent Agreement	98.4%	97.7% - 98.9%		

[a] One ARIES Flu A/B & RSV Assay negative specimen that was positive by the reference 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.
[b] Thirty-two ARIES Flu A/B & RSV Assay positive specimens that were negative by the reference 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 during the study season, the sample set was further supplemented with 40 banked (pre-selected) influenza B positive specimens. The 40 influenza B positive specimens were tested with the ARIES Flu A/B & RSV Assay along with 40 negative clinical specimens in a randomized, blinded fashion. The results from the supplement specimens were analyzed separately from those of the prospective data set. In this retrospective study, seventy-eight (78/80; 97.5%) generated valid influenza B results on the first attempt. Upon repeat testing of two invalid specimens, they yielded valid results. The clinical performance for detection of influenza B in the retrospective study is summarized below.

Influenza B Clinical Performance Summary – Retrospective Specimens

ARIES Flu A/B & RSV Assay	**Reference**			
	Positive	Negative	No Call	TOTAL
Positive	40	0	0	40
Negative	0	40	0	40
No Call	0	0	0	0
Total	40	40	0	80
		95% CI		
Positive Percent Agreement	100.0%	91.2% - 100.0%		
Negative Percent Agreement	100.0 %	91.2% - 100.0%		

4. Clinical cut-off**:**

Please refer to the "Assay cut-off" section of this document.

5. Expected values/Reference range:

The ARIES Flu A/B & RSV Assay positive results (expected values) after allowable re-runs for each individual target are summarized for each enrollment period, stratified by age group and by collection site and presented in four tables below.

Summary of the ARIES Flu A/B & RSV Positive Results by Age Groups for Prospective Clinical Study (January 2015 – March 2015)

Target (Analyte)	Overall (n=1,017)		0-1 year (n=190)		>1-5 years (n=92)		>5-21 years (n=131)		>21-65 years (n=307)		>65 years (n=297)	
	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	No.	Expected Value	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%

Summary of the ARIES Flu A/B & RSV Positive Results by Age Groups for Prospective Clinical Study (November 2015 – February 2016)

Target (Analyte)	Overall (n=1,462)		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%

Summary of the ARIES Flu A/B & RSV Positive Results by Site for Prospective Clinical Study (January 2015 – March 2015)

Target (Analyte)	Overall (n=1,017)		Site 1 (n=238)		Site 2 (n=225)		Site 4 (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%

Summary of the ARIES Flu A/B & RSV Positive Results by Site for Prospective Clinical Study (November 2015 – February 2016)

Target (Analyte)	Overall (n=1,462)		Site 1 (n=573)		Site 2 (n=102)		Site 3 (n=387)		Site 4 (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. Instrument Name:

ARIES System and ARIES M1 System

O. System Descriptions:

1. Modes of Operation:

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

   Yes ___x_____ or No _____

   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:

   A barcode reader may be used for entry of sample IDs, or they may be entered manually.

4. Specimen Sampling and Handling:

   NPS specimens are manually prepared following the user institution's standard procedures and are transferred to an ARIES Flu A/B & RSV assay cassette for analysis.

5. Calibration:

   Calibration is performed by Luminex service personnel using ARIES System Verification Cassettes.

6. Quality Control:

   Each ARIES Flu A/B & RSV assay cassette includes a SPC. The SPC has a known melting temperature (Tm) range and cycle threshold (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.

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.