DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

May 2, 2017

Luminex Corporation
Kate Linak
Regulatory Affairs Scientist
12212 Technology Blvd
Austin, Texas 78727

Re: K163626
Trade/Device Name: ARIES *Bordetella* Assay
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: Class II
Product Code: OZZ
Dated: April 4, 2017
Received: April 4, 2017

Dear Ms. Linak:

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 Part 801 and Part 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 regulation (21 CFR Part 801 and Part 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,

Kristian M. Roth -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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See *PRA Statement below.*

510(k) Number *(if known)*
K163626

Device Name
ARIES® *Bordetella* Assay

Indications for Use *(Describe)*

The ARIES® *Bordetella* Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection and identification of *Bordetella pertussis* (*B. pertussis)* and *Bordetella parapertussis (B. parapertussis)* nucleic acid in nasopharyngeal swab (NPS) specimens obtained from individuals suspected of having a respiratory tract infection attributable to *B. pertussis* or *B. parapertussis*.

The ARIES® *Bordetella* Assay targets the *B. pertussis* toxin promoter and the *B. parapertussis* IS1001 insertion element in the genomes. When clinical factors suggest that *B. pertussis* or *B. parapertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

Negative results for the ARIES® *Bordetella* Assay do not preclude *B. pertussis* or *B. parapertussis* infection and positive results do not rule out co-infections with other respiratory pathogens. The direct detection and identification of *B. pertussis* and *B. parapertussis* nucleic acids from symptomatic patients aids in the diagnosis of *B. pertussis* and *B. parapertussis* respiratory infection in conjunction with other clinical findings and epidemiological information.

The ARIES® *Bordetella* Assay is indicated for use with the ARIES® Systems.

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

[2] Prescription Use (Part 21 CFR 801 Subpart D) O Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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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:

K163626

B. Purpose for Submission:

Clearance of the ARIES® *Bordetella* Assay for use with the ARIES® Systems.

C. Measurand:

Bordetella pertussis toxin promoter, *Bordetella parapertussis* IS1001 insertion element in respective genomes.

D. Type of Test:

Qualitative Real Time Polymerase Chain Reaction (PCR).

E. Applicant:

Luminex Corporation

F. Proprietary and Established Names:

ARIES® *Bordetella* Assay

G. Regulatory Information:

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

H. Intended Use:

1. Intended use(s):

The ARIES® *Bordetella* Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection and identification of *Bordetella pertussis* (*B. pertussis*) and *Bordetella parapertussis* (*B. parapertussis*) nucleic acid in

nasopharyngeal swab (NPS) specimens obtained from individuals suspected of having a respiratory tract infection attributable to *B. pertussis* or *B. parapertussis*.

The ARIES® *Bordetella* Assay targets the *B. pertussis* toxin promoter and the *B. parapertussis* IS1001 insertion element in the genomes. When clinical factors suggest that *B. pertussis* or B*. parapertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

Negative results for the ARIES® *Bordetella* Assay do not preclude *B. pertussis* or *B. parapertussis* infection and positive results do not rule out co-infections with other respiratory pathogens. The direct detection and identification of *B. pertussis* and *B. parapertussis* nucleic acids from symptomatic patients aids in the diagnosis of *B. pertussis* and *B. parapertussis* respiratory infection in conjunction with other clinical findings and epidemiological information.

The ARIES® *Bordetella* 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® Systems.

I. Device Description:

The ARIES® *Bordetella* Assay is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test system that consists of the ARIES® System or the ARIES® M1 System with their included ARIES® Software, an assay-specific cassette, and an assay-specific protocol file. The ARIES® *Bordetella* 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® *Bordetella* Assay cassette directly detects and identifies *B. pertussis* and *B. parapertussis* DNA from nasopharyngeal swab (NPS) specimens collected from the human nasopharynx region.

Nasopharyngeal swab specimens are collected from patients using a commercially available E-Swab™ (Nylon® Flocked Swab along with modified Liquid Amies) or a commercially available nasopharyngeal swab (i.e. rayon, flocked, nylon, plastic shaft, etc.) placed into an approved transport media (i.e UTM, M5, M6, or equivalent). The specimen is then transported to the laboratory for testing. 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® *Bordetella* 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 T_m value of the SPC is used as a reference for determining the target T_m.

The extracted nucleic acid and SPC are transferred via magnetic beads through the cassette to the ARIES® *Bordetella* Assay lyophilized PCR reagents in the PCR tube that contains primer pairs specific to the *B. pertussis* toxin promoter (ptxA-pr), the *B. parapertussis* IS1001 insertion element, and the SPC sequence. Each of the primer pairs are labeled with a distinct fluorophore and detected in distinct channels of the ARIES® Systems. PCR amplification is performed and assay fluorescence is monitored. Incorporation of a quencher-labeled nucleotide results in a decrease in fluorescence for the associated primer pair. Following amplification, the reaction is slowly heated to separate the fluorescent-labeled strand from the quencher-labeled strand, a process that results in an increase in the fluorescence signal. The reaction fluorescence is measured during this process and the temperature at which the change in fluorescence is the maximum T_m of the amplicon. The strands of the amplicons will separate at a specific melting temperature (T_m) and an increase in fluorescence is observed. The instrument fluorescence output is analyzed and test results are determined using the ARIES® System software and the ARIES® *Bordetella* Assay protocol and run files. ARIES® *Bordetella* Assay results may be reported from the ARIES® Software or from the optional SYNCT® Software.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   illumigene® Pertussis DNA Amplification Assay (manufactured by Meridian Bioscience, Inc.)

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

   K133673

3. Comparison with predicate:

   The following table compares the ARIES® *Bordetella* Assay to Meridian Bioscience, Inc.'s *illumigene*® Pertussis DNA Amplification Assay (K133673). 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 (K133673)**
Intended Use	The ARIES® *Bordetella* Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection and identification of *Bordetella pertussis* (*B. pertussis*) and *Bordetella parapertussis* (*B. parapertussis*) nucleic acid in nasopharyngeal swab (NPS) specimens obtained from individuals suspected of having a respiratory tract infection attributable to *B. pertussis* or *B. parapertussis*. The ARIES® *Bordetella* Assay targets the *B. pertussis* toxin promoter and the *B. parapertussis* IS1001 insertion element in the genomes. When clinical factors suggest that *B. pertussis* or B. *parapertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines. Negative results for the ARIES® *Bordetella* Assay do not preclude *B. pertussis* or *B. parapertussis* infection and positive results do not rule out co-infections with other respiratory pathogens. The direct detection and identification of *B. pertussis* and *B. parapertussis* nucleic acids from symptomatic patients aids in the diagnosis of *B. pertussis* and *B. parapertussis* respiratory infection in conjunction with other clinical findings and epidemiological information. The ARIES® *Bordetella* Assay is indicated for use with the ARIES® Systems.	The *illumigene®* Pertussis DNA Amplification Assay, performed on the *illumipro-10™*, is a qualitative in vitro diagnostic test for the direct detection of *Bordetella pertussis* in human nasopharyngeal swab samples taken from patients suspected of having respiratory tract infection attributable to *Bordetella pertussis.* The *illumigene* Pertussis assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect *Bordetella pertussis* by targeting the IS481 insertional element of the *Bordetella pertussis* genome. The IS481 insertional element can also be found in *Bordetella holmesii* and *Bordetella bronchiseptica* strains. Respiratory infection with *Bordetella pertussis*, *Bordetella holmesii* or *Bordetella bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis*. *B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines. Negative results for the *illumi*gene® Pertussis DNA Amplification Assay do not preclude *Bordetella pertussis* infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the *illumigene* Pertussis assay should be used in conjunction with information obtained during the patient's clinical evaluation as an aid in diagnosis of *Bordetella pertussis*

Similarities		
Attribute	**New Device**	**Predicate Device (K133673)**
		infection and should not be used as the sole basis for treatment or other patient management decisions. *illumigene* Pertussis is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.
Sample type	Nasopharyngeal swabs (NPS)	Nasopharyngeal swabs (NPS)
Assay results	Qualitative	Qualitative
Analyte	DNA	DNA

Table 11.2: Differences between New Device and Predicate

Differences		
Attribute	**New Device**	**Predicate Device (K120413)**
Extraction Method	Automated by the ARIES® Systems	Manual extraction
Organisms Detected	*B. pertussis* and *B. parapertussis*	*Bordetella pertussis*
B. pertussis Target	toxin promoter (ptxA-pr)	IS481 Insertional Element
B. parapertussis Target	IS1001 insertion element	N/A
Detection	Different fluorescent reporter dyes for each target and melt analysis. Fluorescence Emissions and Detection	Measurement of magnesium pyrophosphate forming a precipitate in the reaction mixture. Visible Light Transmission
Assay format	Real-time PCR	DNA Amplification; Loop-Mediated Isothermal Amplification (LAMP)
Controls	Internal Control: Sample processing control (SPC)	Internal Control Provided; External positive control included in *illumigene* Pertussis External Control Kit
Instrument	ARIES® System, ARIES® M1 System	*illumipro-10*

K. Standards/Guidance Documents Referenced:

Not applicable.

L. Test Principle:

The ARIES® *Bordetella* 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 melting temperature (T_m) of the amplicon.

M. Performance Characteristics:

1. Analytical performance:

a. Reproducibility/Precision/Repeatability:

Reproducibility of the ARIES® *Bordetella* Assay was evaluated by testing one lot of ARIES® *Bordetella* Assay Cassettes on two ARIES® Systems by two operators at each of three sites, 2 external clinical and 1 internal, on five non-consecutive days. A blinded and randomized reproducibility panel was prepared and sent to these sites by an independent operator that consisted of *B. pertussis* low positive, *B. pertussis* moderate positive, *B. parapertussis* low positive, *B. parapertussis* moderate positive and *B. pertussis* and *B. parapertussis* negative sample diluted into the negative natural nasopharyngeal matrix. Each panel member was tested in triplicate by each operator each day of testing. The results of the reproducibility study are shown in Tables 11.3 and 11.4. The results showed that the reproducibility of the ARIES® *Bordetella* Assay across the three sites met the acceptance criteria of 100% positive for moderate positive samples, ≥ 95% positive for low positive samples, and 100% negative for negative samples.

Table 11.3: ARIES® *Bordetella* Assay Site to Site Reproducibility Results

Targets	Site 1		Site 2		Site 3	
	Positivity		Positivity		Positivity	
***B. pertussis* (Low Positive)**	30/30	100%	30/30	100%	30/30	100%
***B. pertussis* (Moderate Positive)**	30/30	100%	30/30	100%	30/30	100%
***B. parapertussis* (Low Positive)**	30/30	100%	30/30	100%	30/30	100%
***B. parapertussis* (Moderate Positive)**	30/30	100%	30/30	100%	30/30	100%
Negative	0/30	0%	0/30	0%	0/30	0%

Table 11.4: Reproducibility Panel Overall Results

Targets	Positivity		95% Confidence Interval	
			Lower Limit	Upper Limit
***B. pertussis* (Low Positive)**	90/90	100%	96.0%	100.0%
***B. pertussis* (Moderate Positive)**	90/90	100%	96.0%	100.0%
***B. parapertussis* (Low Positive)**	90/90	100%	96.0%	100.0%
***B. parapertussis* (Moderate Positive)**	90/90	100%	96.0%	100.0%
Negative	0/90	0%	0.0%	4.0%

Lot-to-Lot Reproducibility of the ARIES® *Bordetella* Assay was evaluated by one operator using one ARIES® instrument to test three lots of the ARIES® *Bordetella* Assay Cassettes. A reproducibility panel consisting of *B. pertussis* low positive, *B. pertussis* moderate positive, *B. parapertussis* low positive, *B. parapertussis* moderate positive, and *B. pertussis* and *B. parapertussis* negative sample diluted into the negative natural nasopharyngeal matrix were prepared and tested to evaluate the lot-to-lot reproducibility. Three replicates of each sample concentration were run five times (for a total of fifteen replicates) for each lot of the ARIES® *Bordetella* Assay Cassette. The identity of these samples was blinded to the operator. The results of the study are shown in Table 11.5. The test results showed that the reproducibility across the three ARIES® *Bordetella* Assay Cassette lots met the acceptance criteria of 100% positive for moderate positive samples, ≥ 95% positive for low positive samples, and 100% negative for negative samples.

Table 11.5: ARIES® *Bordetella* Assay Lot-to-Lot Reproducibility Determination Results

Target Type	Positivity			
	Lot AA1445	Lot AA1485	Lot AA1505	Overall
***B. pertussis* (Low Positive)**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)
***B. pertussis* (Moderate Positive)**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)
***B. parapertussis* (Low Positive)**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)
***B. parapertussis* (Moderate Positive)**	100% (15/15)	100% (15/15)	100% (15/15)	100% (45/45)
Negative	0% (0/15)	0% (0/15)	0% (0/15)	0% (0/45)

Within-laboratory precision/repeatability for the ARIES® *Bordetella* Assay was evaluated by testing *B. pertussis* and *B. parapertussis* samples at various concentration levels across multiple days utilizing multiple operators, multiple ARIES® Systems and one lot of the ARIES® *Bordetella* Assay Cassette. Study samples were prepared that consisted of *B. pertussis* and *B. parapertussis* culture diluted into negative natural nasopharyngeal matrix at five concentration levels – moderate positive *B. pertussis*, low positive *B. pertussis*, moderate positive *B. parapertussis*, low *positive B. parapertussis*, and a *B. pertussis* and *B. parapertussis* negative sample. These samples were blinded to the operator and tested in triplicate by two operators across five non-consecutive days. The results of the study shown in Table 11.6 demonstrate that results obtained with the ARIES® *Bordetella* Assay between multiple operators using multiple instruments within a laboratory run across multiple days are repeatable across a range of *Bordetella* concentration levels.

Table 11.6: ARIES® *Bordetella* Assay Within Laboratory Precision/ Repeatability Results

Target Type	Expected Positivity	Positivity	95% Confidence Interval
***B. pertussis* (Low Positive)**	Approximately 95%	100% (30/30)	88.4% - 100.0%
***B. pertussis* (Moderate Positive)**	100%	100% (30/30)	88.4% - 100.0%
***B. parapertussis* (Low Positive)**	Approximately 95%	100% (30/30)	88.4% - 100.0%
***B. parapertussis* (Moderate Positive)**	100%	100% (30/30)	88.4% - 100.0%
Negative	0%	0% (0/30)	0.0% - 11.6%

b. Linearity/assay reportable range:

Not applicable. The ARIES® *Bordetella* Assay is a qualitative assay.

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

Stability:

Specimen Stability (Fresh vs. Frozen)

The performance equivalency of the ARIES® *Bordetella* Assay was assessed using contrived *Bordetella* specimens when tested from the fresh state (i.e. unfrozen) and specimens that were tested after being stored at -65 to -95°C (frozen). Equivalency was evaluated using samples consisting of *Bordetella pertussis* and *Bordetella parapertussis* culture diluted independently into natural negative human nasopharyngeal swab matrix at three test concentrations. A total of 120 samples were tested for each *Bordetella* strain of which half were tested immediately upon preparation (fresh) and the remaining samples were frozen at -65 to -95°C and tested 24 to 48 hours later. Six replicates of natural negative human nasopharyngeal swab matrix were also tested fresh and frozen as a negative control. Of each set of fresh and frozen samples, 50% were targeted to 3x LoD, 25% were targeted to 10X LoD and the remaining 25% of the samples were targeted to 100X LoD.

The results of the study are shown in Table 11.7. Based on this study, no significant difference in the performance of ARIES® *Bordetella* Assay was observed between specimens tested fresh and specimens that were tested after being stored frozen at -65 to -95°C.

Table 11.7: ARIES® *Bordetella* Assay Fresh vs. Frozen Contrived Specimen Stability Results

Target type	Concentrations	Agreement with Expected Results	
		Fresh Samples	Frozen Samples
B. pertussis	**3x LoD**	100% (30/30)	100% (30/30)
	10x LoD	100% (15/15)	100% (15/15)
	100x LoD	100% (15/15)	100% (15/15)
B. parapertussis	**3x LoD**	100% (30/30)	100% (30/30)
	10x LoD	100% (15/15)	100% (15/15)
	100x LoD	100% (15/15)	100% (15/15)
Natural Negative Matrix	**Negative**	100% (6/6)	100% (6/6)

Shelf-Life Stability

A real time stability study was performed to evaluate the shelf life of ARIES® *Bordetella* Assay Cassettes. Stability was assessed by testing three replicates of *Bordetella* Extractable Control, 100x LoD Blend and three replicates of negative targets (Copan UTM) on three different lots of ARIES® *Bordetella* cassettes stored at two different temperatures (4°C (2 – 8

°C) and room temperature (15 – 30 °C)). The study was designed following guidelines listed in EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline and BS EN ISO 23640: 2013 - In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents. Acceptance criteria for stability at each time point and temperature were established as 100% positivity for all *Bordetella* replicates and 100% negativity for all negative replicates. Data for ARIES® *Bordetella* Assay Cassette stability has been collected up to 7 months and gave expected results indicating stability up to 7 months. Stability studies are on-going.

Controls:

Process Control

Each ARIES® *Bordetella* 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. Reference *Bordetella pertussis* and *Bordetella parapertussis* strains or well characterized *Bordetella pertussis* and *Bordetella parapertussis* clinical isolates may be used as positive controls. The ARIES® *Bordetella* Assay Cassette Kit does not include external positive and negative controls.

d. Detection Limit:

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES® *Bordetella* Assay using two strains each of *B. pertussis* and *B. parapertussis* diluted in natural negative nasopharyngeal (NP) swab matrix. Preliminary LoD concentrations were determined using serial dilutions of each *Bordetella* strain in natural negative nasopharyngeal (NP) swab matrix where each dilution was quantified using standard dilution and quantitative culture techniques. These preliminary LoD concentrations were confirmed by testing twenty (20) replicates of each strain. All *Bordetella* strain concentrations were verified by plating and colony counting (CFU/mL). The LoD for each *Bordetella* strain was determined as the lowest concentration that had a positivity rate of ≥ 95%. The final LoD concentrations for the four strains of *Bordetella* are shown in Table 11.8. The overall assay LoD for *B. pertussis* is 1,800 CFU/mL and *B. parapertussis* is 213 CFU/mL.

Table 11.8: ARIES® *Bordetella* Assay Limit of Detection Results

Bordetella Type	Strain	Concentration (CFU/mL)	Positivity	95% Confidence Interval
B. pertussis	A639	1,640	95% (19/20)	75.1% - 99.9%
	BAA- 589	1,800	95% (19/20)	75.1% - 99.9%
B. parapertussis	A747	172	100% (20/20)	83.2% - 100.0%
	BAA-587	213	95% (19/20)	75.1% - 99.9%

e. Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the ARIES® *Bordetella* Assay was evaluated against eighteen (18) *Bordetella* strains; eleven *B. pertussis* and seven *B. parapertussis* strains. These strains differed from those that were tested as part of the Limit of Detection study. The specimens were prepared by diluting quantified cultured organism into pooled natural negative human nasopharyngeal swab matrix at a concentration of three times the confirmed limit of detection of the ARIES® *Bordetella* Assay and tested in triplicate. All seven strains of *B. parapertussis* were detected with 100% positivity at 3x LoD. Additionally, nine of the eleven strains of *B. pertussis* were detected with 100% positivity at 3x LoD while two strains, ATCC 8478 and ATCC 9797, were not detected at either 3x, 10x or 100x LoD. Sequencing of these two strains showed that both strains contained a similar nucleotide mismatch in both ARIES® *B. pertussis* primer binding regions, forward and reverse, which may impact the ability of the ARIES® *Bordetella* assay to detect these strains. An *in-silico* search of *Bordetella pertussis* sequences in the NCBI database identified a low prevalence of strains with similar mismatches (2.9%). Furthermore, all of these strains have decades old collection dates for strains with known human hosts—suggesting that these strains are no longer prevalent in the human population. Thus, there is a low risk of the ARIES® *Bordetella* Assay missing a circulating strain of *B. pertussis*. The results of the inclusivity testing are shown in Table 11.9.

Table 11.9: ARIES® *Bordetella* Assay Analytical Reactivity (Inclusivity) Results

Inclusivity Strains	Test Concentration[b]	Result	Positivity
ATCC BAA-1335	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
ATCC 8467	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
ATCC 12742	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
ATCC 12743	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
ATCC 51445	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
E431	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
ATCC 10380	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
NR-42457	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
NR-42460	5,400 CFU/mL	*B. pertussis* Detected	100% (3/3)
ATCC 8478	180,000 CFU/mL[a]	*B. pertussis* Not Detected	0% (3/3)
ATCC 9797	180,000 CFU/mL[a]	*B. pertussis* Not Detected	0% (3/3)

Inclusivity Strains	Test Concentration[b]	Result	Positivity
ATCC 9305	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)
ATCC 15237	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)
ATCC 15311	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)
ATCC 15989	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)
C510	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)
E595	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)
E838	639 CFU/mL	*B. parapertussis* Detected	100% (3/3)

[a] Two strains of *B. pertussis*, ATCC 8478 and ATCC 9797 were not detected using the ARIES® *Bordetella* Assay up to 100x of the assay LoD.
[b] *B. pertussis* and *B. parapertussis* strains were tested at 3x LoD.

f. Analytical specificity:

Interfering Substances:

The potential inhibitory effect of non-microbial substances expected to be found in human nasopharyngeal swab specimens was evaluated for the ARIES® *Bordetella* Assay. Three replicates each of *Bordetella pertussis* and *Bordetella parapertussis* were tested at concentrations near the assay LoD with a relative, high concentration of each non-microbial substance spiked into the contrived sample. Additionally, negative natural human nasopharyngeal swab matrix was spiked with the same concentration of each non-microbial substance and tested for assay interference. *Bordetella pertussis* and *Bordetella parapertussis* samples were contrived by independently diluting cultures into natural negative human nasopharyngeal swab matrix at a concentration of three times the limit of detection of the ARIES® *Bordetella* Assay for the strain tested.

The results of the study are shown in Table 11.10. Based on this study, none of the substances tested showed any interference with the ARIES® *Bordetella* Assay.

Table 11.10: ARIES® *Bordetella* Assay Interfering Substance Results

Interfering Substance	Test Concentration
Benzocaine	2.5% (w/v)
Budesonide	25 mg/mL
Dexamethasone	3 mg/mL
Flunisolide	55 mg/mL
Fluticasone (Nasal Corticosteroids)	5% (v/v)
FluMist®	10% (v/v)
Human Blood (EDTA)	5% (v/v)
Menthol	0.26% (w/v)
Mometasone	2.5 mg/mL
Mucin protein	1% (w/v)
Mupirocin	2% (w/v)
Oseltamivir Phosphate (Anti-viral drugs)	10 mg/mL
Oxymetazoline nasal spray (Afrin®)	15% (v/v)
Phenylephrine	0.3 mg/mL
Smokeless tobacco	1% (w/v)

Interfering Substance	Test Concentration
Sodium chloride	0.0065% (w/v)
Tobramycin (Antibacterial, systemic)	0.6 mg/mL
Triamcinolone	5.5 µg/mL
Zanamivir (Anti-viral drugs)	5 mg/mL
Zicam Nasal gel (Histaminum hydrochoricum, Galphimia glauca, Luffa opperculata, Sulfur)	5% (v/v)

Cross-Reactivity (Exclusivity):

Cross reactivity for the ARIES® *Bordetella* Assay was assessed with 65 unique microorganisms that are common to the same human matrix as *B. pertussis* and or *B. parapertussis* infections, that cause infections that might present similar symptoms to *B. pertussis* and or *B. parapertussis* infections, and that could potentially interfere with the diagnostic capabilities of the ARIES® *Bordetella* Assay. Bacteria were tested at $\geq 10^6$ CFU/mL or the highest available concentration, and viruses were tested at $\geq 10^5$ $TCID_{50}$/mL or the highest available concentration. These potential cross reactive organisms were spiked at high concentration into natural negative human nasopharyngeal swab matrix and tested in triplicate (n=3) on the ARIES® System. In addition to the 65 unique microorganisms, multiple strains of *Bordetella bronchiseptica* and *Bordetella holmesii* were also tested for cross-reactivity for a total of 71 microorganisms. Of the 71 microorganisms tested, 66 yielded negative results for *B. pertussis* and *B. parapertussis* and thus are considered non-reactive with the ARIES® *Bordetella* Assay. Five organisms, *Fusobacterium necrophorum*, Human Coronavirus OC43, Influenza B, *Moraxella catarrhalis* and *Proteus vulgaris* generated a false positive result in 1 out of 6 replicates. All microorganisms evaluated are listed in Table 11.11.

Microbial Interference (Cross Reactivity)/Co-Infection:

Microbial interference for the ARIES® *Bordetella* Assay was assessed with 65 unique cross reactive microorganisms (CRO) that are commonly found in the same human matrix as *B. pertussis* and or *B. parapertussis* infections, that cause infections that might present similar symptoms to *B. pertussis* and or *B. parapertussis* infections, and could potentially interfere with the diagnostic capabilities of the ARIES® *Bordetella* Assay. Bacteria were tested at $\geq 10^6$ CFU/mL or the highest available concentration, and viruses were tested at $\geq 10^5$ $TCID_{50}$/mL or the highest available concentration. The potential interfering organisms were spiked into natural negative human nasopharyngeal swab matrix containing representative strains of *Bordetella pertussis* (BP) or *Bordetella parapertussis* (BPP) near the LoD concentration. All target strain + CRO samples were tested in triplicate (n=3) on the ARIES® System. In addition to the 65 unique microorganisms, multiple strains of *Bordetella bronchiseptica* and *Bordetella holmesii* were also tested for microbial interference. *B. pertussis* was correctly detected in 3/3 replicates when tested in the presence of 66 CROs, with 5 CROs, *Bordetella bronchiseptica* (strain 1 and strain 2), *Bordetella petrii, Enterobacter aerogenes, Klebsiella pneumoniae* requiring additional testing of 3 replicates per protocol. For these 5 CROs, *B.*

pertussis was detected in 5/6 replicates. *B. parapertussis* was correctly detected in 3/3 replicates when tested in the presence of all 71 CROs.

Microbial interference was also evaluated in a co-infection setting where near LoD concentration *B. pertussis* was tested with high concentration of *B. parapertussis* and vice-versa. A low/low and a high/high combination of *B. pertussis* and *B. parapertussis* were also evaluated. All replicates in all combinations yielded expected positivity for *B. pertussis* and *B. parapertussis.* All microorganisms evaluated are listed in Table 11.11.

Table 11.11: Microorganism Information

Microorganism			
1	*Acinetobacter baumanii*	*37*	Human Coronavirus 229E
2	*Acinetobacter calcoaceticus*	*38*	Human Coronavirus OC43[3]
3	*Acinetobacter Iwoffii*[9]	*39*	Influenza A
4	Adenovirus 7A	*40*	Influenza B[3]
5	Adenovirus Type 1	*41*	*Klebsiella pneumoniae*[4]
6	Adenovirus Type 3	*42*	*Lactobacillus acidophilus*
7	*Arcanobacterium haemolyticum*[9]	*43*	*Lactobacillus plantarum*
8	*Bacteroides fragilis*	*44*	*Legionella pneumophila*
9	*Bordetella avium*	*45*	Metapneumovirus hMPV 20 Type A2
10	*Bordetella bronchiseptica (ATCC 19395)*[4,5,10]	*46*	Metapneumovirus hMPV 8 Type B2
11	*Bordetella bronchiseptica (ATCC 4617)*[4,6,10]	*47*	*Moraxella catarrhalis*[3]
12	*Bordetella bronchiseptica (ATCC BAA-588)*	*48*	*Morganella morganii*
13	*Bordetella bronchiseptica (Clinical Isolate)*	*49*	Mumps virus
14	*Bordetella hinzii*	*50*	*Mycobacterium tuberculosis*
15	*Bordetella holmesii (F061)*	*51*	*Mycoplasma pneumoniae*
16	*Bordetella holmesii (C690)*	*52*	*Neisseria elongata*
17	*Bordetella holmesii (ATCC 51541)*[9]	*53*	*Neisseria meningitidis*
18	*Bordetella holmesii (NCTC 13202)*	*54*	*Oligella ureolytica*
19	*Bordetella petrii*[4]	*55*	Parainfluenza Type 1
20	*Bordetella trematum*	*56*	Parainfluenza Type 2
21	*Burkholderia cepacia*	*57*	Parainfluenza Type 3
22	*Candida albicans*[7,11]	*58*	*Parvimonas micra*[1]
23	*Chlamydophila pneumoniae*	*59*	*Proteus vulgaris*[3]
24	*Citrobacter freundii*	*60*	*Pseudomonas aeruginosa*[9]
25	*Corynebacterium diptheriae*	*61*	*Ralstonia paucula*[2]
26	Coxsackievirus[9]	*62*	Respiratory Syncytial Virus
27	Cytomegalovirus	*63*	Rhinovirus
28	Echovirus	*64*	*Staphyloccocus aureus*
29	*Enterobacter aerogenes*[4]	*65*	*Staphyloccocus aureus (MRSA)*
30	*Enterococcus faecalis*	*66*	*Staphylococcus epidermidis*
31	Enterovirus	*67*	*Staphylococcus hominis*
32	Epstein-Barr virus	*68*	*Stenotrophomonas maltophilia*

Microorganism			
33	*Escherichia coli*	69	*Streptococcus pneumoniae*
34	*Fusobacterium necrophorum*[3]	70	*Streptococcus pyogenes*
35	*Haemophilus influenzae*	71	*Streptococcus salivarius*
36	Human Bocavirus	72	*Bordetella pertussis*[8]
		73	*Bordetella parapertussis*[8]

[1] Formerly *Micromonas micros* and *Peptostreptococcus micros*

[2] Formerly *Cupriavidus pauculus*

[3] A false positive result was observed in 1 out of 6 replicates during cross-reactivity testing.

[4] *B. pertussis* was detected in 5/6 replicates during microbial interference studies.

[5] *B. parapertussis (BPP)* was not detected in one of the three replicates of BPP+CRO when CRO was spiked at $1.35x10^8$ CFU/mL. However, *B. parapertussis* was detected in all replicates when the CRO concentration was reduced to 10^6 CFU/mL. All replicates of *B. pertussis* were detected when CRO was spiked at $1.35x10^8$ CFU/mL.

[6] *B. pertussis (BP)* was not detected in one of the three replicates of BP+CRO when CRO was spiked at $3.64x10^8$ CFU/mL. However, *B. pertussis* was detected in all replicates when the CRO concentration was reduced to 10^6 CFU/mL. All replicates of *B. parapertussis* were detected when CRO was spiked at $3.64x10^8$ CFU/mL.

[7] A false positive for *B. parapertussis* was detected in one of the three replicates of BP+CRO when CRO was spiked at $8.7x10^6$ CFU/mL. However, no false positive for *B. parapertussis* was detected when the CRO concentration was reduced to 10^6 CFU/mL.

[8] High concentration of *B. pertussis* (≥ 10^6 cfu/mL) was tested with low concentration of *B. parapertussis* (3x LoD) and vice-versa to evaluate the impact of microbial interference in a co-infection setting, during cross-reactivity studies. *B. pertussis* and *B. parapertussis* were also tested in a low/low, high/high setting, where low concentration was 3x LOD and high concentration was 100x LOD.

[9] A total of 6 replicates were tested with 1 out of 6 replicates resulting in a false positive result for *B. parapertussis*, during microbial interference studies.

[10] Initial testing of 3 replicates resulted in a false negative for *B. pertussis* or *B. parapertussis*, during microbial interference studies. Subsequent repeat testing yielded the same outcome. After concentration of the CRO was reduced to 10^6 CFU/mL, expected positivity was achieved.

[11] Initial testing of 3 replicates resulted in a false positive for *B. parapertussis*, during microbial interference studies. Subsequent repeat testing yielded the same outcome. After concentration of the CRO was reduced to 10^6 CFU/mL, expected positivity was achieved.

Carry-Over/Cross-Contamination:

Carry-over and cross contamination for the ARIES® *Bordetella* Assay was evaluated by using samples consisting of *Bordetella pertussis* culture diluted into pooled natural negative human nasopharyngeal swab matrix at a concentration of $1x10^6$ CFU/mL. Testing was performed using thirty high positive *Bordetella pertussis* samples in series alternating with thirty *Bordetella pertussis and Bordetella parapertussis* negative (pooled natural negative human nasopharyngeal swab matrix) samples. The high positive samples were run adjacent to negative samples across five consecutive runs using one ARIES® System.

The result of the study was no carry-over or cross contamination observed.

g. Assay cut-off:

Each target in the ARIES® *Bordetella* assay (*B. pertussis* and *B. parapertussis*) has a Ct cut-off, Tm window, and Tm Peak Threshold. In addition, the internal sample process control (SPC) also has a corresponding Ct cut-off, Tm window, and Tm Peak Threshold. Collectively, the cut-off values compose the assay protocol file parameters, which are used to determine the assay result for the detection target as POSITIVE, NEGATIVE, or INVALID.

The Assay Protocol File parameters were determined, and their performance in the ARIES® *Bordetella* Assay were evaluated according to the following general procedure:

- Initial Assay Protocol File parameters were set during internal optimization studies
- The final Assay Protocol File parameters were then established during internal verification studies
- The selected Assay Protocol File parameter values were utilized in the determination of assay performance in the multi-site clinical trial conducted for the ARIES® *Bordetella* Assay

2. Comparison Studies:

a. Method comparison with predicate device:

Not applicable.

b. Collection Media Comparison:

The compatibility of four media, UTM™, M5®, M6™ and ESwab™, was evaluated with the ARIES® *Bordetella* Assay using two strains each of *Bordetella pertussis* and *Bordetella parapertussis.* Each strain of *Bordetella* was independently diluted in the media at a concentration of three times the limit of detection of the ARIES® *Bordetella* Assay for that strain. The study was performed in multiple assay runs with twenty replicates for each media type and each strain of *Bordetella* and three replicates for each media type by itself as the negative control.

The results of the study are shown in Table 11.12. Based on this study, the four different types of media when tested with the two different types of *Bordetella* strains were determined to be compatible with the ARIES® *Bordetella* Assay.

Table 11.12: ARIES® *Bordetella* Assay Media Equivalency Results

Concentration/ Target type	Media Type	Mean Ct ± SD (cycles)	% Positivity/ Detected
***3x LoD B. pertussis* (A639)**	**M6**	35.8 ± 0.96	100% (20/20)
	M5	35.6 ± 0.66	100% (20/20)
	Universal Transport Media (UTM)	36.4 ± 0.86	100% (20/20)
	ESwab (Modified Liquid Amies)	36.2 ± 0.75	100% (20/20)
***3x LoD B. pertussis* (BAA-589)**	**M6**	35.9 ± 0.67	100% (20/20)
	M5	36.7 ± 1.03	100% (20/20)
	Universal Transport Media (UTM)	37.0 ± 0.76	95% (19/20)
	ESwab (Modified Liquid Amies)	36.0 ± 0.68	100% (20/20)
***3x LoD B. parapertussis* (A747)**	**M6**	33.8 ± 0.53	100% (20/20)
	M5	33.6 ± 0.79	100% (20/20)
	Universal Transport Media (UTM)	34.9 ± 0.62	100% (20/20)
	ESwab (Modified Liquid Amies)	34.9 ± 1.05	100% (20/20)
***3x LoD B. parapertussis* (BAA-587)**	**M6**	33.8 ± 0.58	100% (20/20)
	M5	33.0 ± 0.67	100% (20/20)
	Universal Transport Media (UTM)	34.7 ± 0.83	100% (20/20)
	ESwab (Modified Liquid Amies)	33.9 ± 0.54	100% (20/20)
[a]No Target	**M6**	[a]29.9 ± 0.20	100% (3/3)
	M5	[a]30.0 ± 0.40	100% (3/3)
	Universal Transport Media (UTM)	[a]29.4 ± 0.36	100% (3/3)
	ESwab (Modified Liquid Amies)	[a]30.2 ± 0.06	100% (3/3)

[a] Data shown for DNA SPC

c. Swab Comparison:

The compatibility of 3 different types of nasopharyngeal swabs, Flocked, Rayon and Polyester was evaluated with the ARIES® *Bordetella* Assay using one strain each of *Bordetella pertussis* and *Bordetella parapertussis.* The *Bordetella* strains were diluted independently into pooled natural negative human nasopharyngeal swab matrix and applied to each swab type. The collection swabs were then inserted and broken off inside 3mL Universal Transport Medium (UTM) vials for a final concentration of 3x LoD in the transport medium. In addition, a negative sample consisting of the swab only transferred in UTM was also evaluated.

The results of the study are shown in Table 11.13. Based on this study, the three different types of nasopharyngeal swabs when tested with the *Bordetella* strains were determined to be compatible with the ARIES® *Bordetella* Assay.

Table 11.13: ARIES® *Bordetella* Assay Nasopharyngeal Swab Equivalency Results

Concentration/ Target type	Swab Type	Mean Ct ± SD (cycles)	% Positivity/ Detected
***3x LoD B. pertussis* (A639)**	**Flocked Swab**	38.4 ± 1.33	100% (9/9)
	Polyester Swab	36.9 ± 0.75	100% (9/9)
	Rayon Swab	37.3 ± 1.53	100% (9/9)
	No Swab (Positive Control)	37.6 ± 1.04	100% (3/3)
***3x LoD B. parapertussis* (A747)**	**Flocked Swab**	35.4 ± 0.87	100% (9/9)
	Polyester Swab	33.8 ± 0.92	100% (9/9)
	Rayon Swab	34.1 ± 1.18	100% (9/9)
	No Swab (Positive Control)	34.2 ± 0.90	100% (3/3)
[a]No Target	**Flocked Swab**	[a]31.8 ± 0.45	100% (3/3)
	Polyester Swab	[a]31.1 ± 0.21	100% (3/3)
	Rayon Swab	[a]31.3 ± 0.42	100% (3/3)

[a] Data shown for DNA SPC

3. Clinical Performance:

The clinical performance of ARIES® *Bordetella* Assay was evaluated using leftover de-identified nasopharyngeal swab (NPS) specimens prospectively collected from pediatric and adult patients suspected of having respiratory tract infection attributable to *B. pertussis* or *B. parapertussis.*

Five (5) geographically distinct clinical sites within the United States prospectively collected specimens from July through November 2016. The clinical performance of the ARIES® *Bordetella* Assay was evaluated on these prospectively collected specimens at four (4) of the five (5) clinical sites using the ARIES® System. One (1) clinical site collected specimens then shipped them frozen on dry ice to one of the other clinical sites for ARIES® *Bordetella* Assay testing. Specimens included in the clinical study consisted of leftover de-identified nasopharyngeal swabs (NPS) specimens prospectively collected from pediatric and adult patients suspected of having respiratory tract infection attributable to *B. pertussis* or *B. parapertussis.*

A total of 1052 unique specimens that met the pre-determined inclusion criteria were included in the study and tested for *B. pertussis* or *B. parapertussis* by both the reference method and the ARIES® *Bordetella* Assay. The performance of ARIES® *Bordetella* Assay for *B. pertussis* and *B. parapertussis* was compared to a composite comparator assay consisting of two well-characterized real-time PCR assays (for each bacterial pathogen) followed by confirmation of positive PCR amplification product with bi-directional sequencing. Comparator PCR assays for *B. pertussis* and *B. parapertussis* targeted unique sequences within the promoter region of the ptxA gene and IS1001 insertion region (respectively) that were different than those targeted by the ARIES® *Bordetella* Assay. Specimens were characterized as positive for *B. pertussis* or *B. parapertussis* if one out of two comparator PCR assays was positive (Ct values ≤40) and confirmed by bi-directional sequencing, or if

both comparator PCR assays were positive. Specimens were characterized as *B. pertussis* or *B. parapertussis* negative if one out of two comparator PCR assays was negative (Ct values >40) and confirmed by bi-directional sequencing, or if both comparator PCR assays were negative.

Comparator real-time PCR and bi-directional sequencing assays were performed at a centralized testing facility. Clinical runs and re-runs using ARIES® *Bordetella* Assay were carried out by trained operators at the four (4) testing sites on specimens that were either kept refrigerated at 2°C to 8°C for up to 72 hours prior to testing (N=667; 63.4%) or stored frozen at -65°C to -95°C for up to 12 days prior to testing (N=385; 36.6%).

Out of the 1052 clinical specimens included in the prospective study analysis, 1043 (99.1%) generated valid ARIES® *Bordetella* Assay results (i.e., positive or negative) on the first attempt. There were 9 specimens (9/1052; 0.9%) that were re-tested with ARIES® *Bordetella* Assay because they yielded invalid results in the initial run (N=3) or because of instrument error (N=6). All nine (9) specimens generated valid ARIES® results upon repeat testing.

In the prospective study, the ARIES® *Bordetella* Assay Positive Percent Agreement (PPA) for *B. pertussis* was reported to be 93.8% (30/32) with a lower bound of the 95% confidence interval of 79.2%. Positive Percent Agreement of the ARIES® *Bordetella* Assay for *B. parapertussis* was reported to be 100% (2/2) with a lower bound of the 95% confidence interval of 15.8%. Negative Percent Agreement of the ARIES® *Bordetella* Assay for *B. pertussis* and *B. parapertussis* were respectively; 98.9% (1009/1020) with a lower bound 95% confidence interval of 98.1%, and 99.8% (1048/1050) with a lower bound 95% confidence interval of 99.3%.

Due to the low prevalence *B. pertussis* and *B. parapertussis* observed in the prospective study, the clinical sample set was supplemented with banked (pre-selected) *B. pertussis* (N=37) and *B. parapertussis* (N=20) positive specimens as well as contrived *B. parapertussis* specimens (N=50). Pre-selected *B. pertussis* specimens were collected at six (6) clinical sites in the United States while pre-selected *B. parapertussis* specimens were collected at three (3) sites also located in the United States. Contrived *B. parapertussis* samples were prepared by spiking well-characterized bacterial strains into individual negative clinical samples (NP swabs) at clinically relevant titers. *B. parapertussis* contrived specimens were prepared at analyte concentrations near the ARIES® *Bordetella* Assay limit of detection (LoD) as well as concentrations spanning the clinically moderate to high positive ranges. The presence of the expected bacterial target in each of the pre-selected and contrived specimens was confirmed by comparator real-time PCR and bi-directional sequencing assays. In addition, bacterial organism concentrations in contrived specimens were verified by culture methods (plating and colony count). In order to minimize bias, pre-selected and contrived specimens were tested along with an equal number of unique negative clinical specimens in a randomized, blinded fashion at three (3) external testing sites. ARIES® *Bordetella* Assay accurately detected all 37 *B. pertussis* (100% PPA; 95% confidence interval: 90.5% - 100%) and all 20 *B. parapertussis* positive specimens tested (100% PPA; 95% confidence interval: 83.2% - 100%). One of the pre-selected specimens generated a false

positive result for *B. parapertussis* by ARIES® *Bordetella* Assay when compared to the composite comparator method (01-122). All fifty (50) contrived *B. parapertussis* samples were accurately detected by the ARIES® *Bordetella* assay (100% PPA; 95% confidence interval 92.9%- 100%). All but one of the pre-selected and contrived specimens generated valid ARIES® result upon initial testing. The invalid result was resolved upon re-test.

The performance of the ARIES® *Bordetella* Assay for *B. pertussis* and *B. parapertussis* as compared to the composite reference method is summarized in the Table 11.14 and Table 11.15 for prospective, pre-selected and contrived specimens. The overall performance of the ARIES® *Bordetella* Assay in combined prospective, pre-selected and contrived specimens is also presented.

Table 11.14: ARIES® *Bordetella* Assay Performance for *B. pertussis*

Specimen Description	PPA		95% CI	NPA		95% CI
Prospective	30/32[1]	93.8%	79.2% - 99.2%	1009/1020	98.9%	98.1% - 99.5%
Pre-selected	37/37	100%	90.5% - 100%	77/77	100%	95.3% - 100%
Total	67/69	97.1%	89.9% - 99.6%	1086/1097	99.0%	98.2% - 99.5%

[1] Two (2) prospective specimens generated false negative results by ARIES® *Bordetella* assay when compared to the composite comparator method (02-179 and 06-267).

Table 11.15: ARIES® *Bordetella* Assay Performance for *B. parapertussis*

Specimen Description	PPA		95% CI	NPA		95% CI
Prospective	2/2	100%	15.8% - 100%	1048/1050	99.8%	99.3% - 100%
Pre-selected	20/20	100%	83.2% - 100%)	93/94[1]	98.9%	94.2% - 100%
Contrived	50/50	100%	92.9% - 100%	50/50	100%	92.9% - 100%
Total	72/72	100%	95.0% - 100%	1191/1194	99.7%	99.3% - 99.9%

[1] One (1) pre-selected specimen generated a false positive result by ARIES® *Bordetella* Assay when compared to the composite comparator method (01-122).

The study results demonstrate that the diagnostic accuracy of ARIES® *Bordetella* Assay is acceptable for the safe and effective detection of *B. pertussis* and *B. parapertussis* in nasopharyngeal swabs (NPS) specimens from patients suspected of having respiratory tract infection attributable to *B. pertussis* or *B. parapertussis*.

4. Expected values:

The overall prevalence of *B. pertussis* and *B. parapertussis,* as reported by the ARIES® *Bordetella* Assay, in prospectively collected symptomatic clinical specimens during the enrollment period was 3.9% (41/1052) and 0.4% (4/1052) respectively.

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.