Luminex

Package Insert | IVD

ARIES® *Bordetella* Assay

IVD For *In Vitro* Diagnostic Use.

For Use With ARIES® Systems.

Rx Only

Luminex Corporation
12212 Technology Blvd.
Austin, Texas 78727
U.S.A.

Technical Support

Telephone: 512-381-4397
North America Toll Free: 1-877-785-2323
International Toll Free: + 800-2939-4959
Email: *support@luminexcorp.com*
www.luminexcorp.com

ARIES® *Bordetella* Assay Package Insert

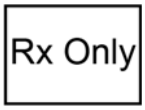
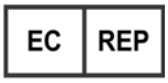
89-30000-00-574 Rev A
May 2017

WMDE
Bergerweg 18
6085 AT Horn
The Netherlands

Luminex Corporation (Luminex) reserves the right to modify its products and services at any time. Notifications will be sent to end users regarding changes that impact the use, performance and /or safety and effectiveness of the device. Any modifications to the device will be made in accordance with applicable regulatory requirements. Luminex assumes no liability for any damages resulting from the off-label application or misuse of this information.

Key to Symbols

5.1.4* 	Use-by date Indicates the date after which the medical device is not to be used.	5.2.8* 	Do not use if package is damaged. Indicates a medical device that should not be used if the package has been damaged or opened.
5.1.5* 	Batch Code Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.3.7* 	Temperature Limit Indicates the temperature limits to which the medical device can be safely exposed.
5.1.6* 	Catalog(ue) Number Indicates the manufacturer's catalogue number so that the medical device can be identified.	5.4.4* 	Caution. Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
5.1.1* 	Manufacturer Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.	5.5.5* 	Contains Sufficient for <n> Tests Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
5.4.3* 	Consult instructions for use. Indicates the need for the user to consult the instructions for use.	5.4.1* 	Biological risks Indicates that there are potential biological risks associated with the medical device.
BC	Build Code	GHS02~ 	Highly flammable liquid and vapor
5.4.2* 	Do not reuse Indicates a medical device that is intended for one use, or for use on a single patient during a single procedure.	5.5.1* 	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

% 	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner (U.S. Only). 21 CFR 809 (FDA Code of Federal Regulations)	# 	Conformite Europeenne (EU CE Marking of Conformity) CE conformity marking
5.1.2* 	Authorized representative in the European Community Indicates the Authorized representative in the European Community		

* ANSI/AAMI/ISO 15223-1:2012, Medical devices—Symbols to be used with medical devices labels, labeling, and information to be supplied—Part 1: General requirements.

~ ST/SG/AC.10/30/Rev.6 Globally Harmonized System of Classification and Labeling of Chemicals (GHS) Sixth revised edition.

Council Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDMD) (1998)

% 21 CFR 809 (FDA Code of Federal Regulations)

Standard Terms and Conditions For Use of Assay Product

By opening the packaging containing this assay product or cassette ("Product") or by using such Product in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return the Product for a full refund prior to using them in any manner.

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strict compliance with the United States Food and Drug Administration and all applicable domestic and international laws and regulations, now and hereinafter enacted.

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Buyer has the responsibility and hereby expressly assumes the risk to verify the hazards and to conduct any further research necessary to learn the hazards involved in using the Product. Buyer also has the duty to warn Buyer's customers, employees, agents, assigns, officers, successors and any auxiliary or third party personnel (such as freight handlers, etc.) of any and all risks involved in using or handling the Product. Buyer agrees to comply with instructions, if any, furnished by Seller or Luminex relating to the use of the Product and to not misuse the Product in any manner. Buyer shall not reverse engineer, decompile, disassemble or modify the Product. Buyer acknowledges that Luminex retains ownership of all patents, trademarks, trade secrets and other proprietary rights relating to or residing in the Product and Buyer receives no rights to such intellectual property rights by virtue of its purchase of Product other than as expressly set forth herein. Buyer shall have no right to use any trademarks owned or licensed to Luminex without the express written permission of Luminex.

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89-30000-00-187 Rev C

Table of Contents

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.

Summary and Explanation of the Test

Pertussis, commonly known as whooping cough, is a contagious respiratory disease caused by the organism *Bordetella pertussis*. *B. pertussis* can lead to life-threatening complications in infants and young children. Since the availability and widespread use of the pertussis vaccine in the 1940s, the disease prevalence has decreased more than 75% compared with the pre-vaccine era. In spite of widespread childhood vaccination, over 100,000 cases were reported in the United States between 2012 and 2014 (CDC — Pertussis Cases by Year (1922-2014)). Studies have estimated the number of *B. pertussis* related cough illnesses to be between 800,000 to 3.3 million per year in the United States (Cherry 2005). WHO estimated 16 million pertussis cases worldwide with 195,000 estimated deaths in children due to the disease (WHO 2010).

Another species of *Bordetella*, *Bordetella parapertussis*, also causes respiratory disease in humans. *B. parapertussis* infections present with symptoms similar to whooping cough and may be responsible for up to 20% of pertussis-like disease, mainly in young children (Mattoo and Cherry 2005). *B. parapertussis* tends to produce milder disease than *B. pertussis*. There is no cross-reactivity between the pertussis vaccine and *B. parapertussis*; therefore all populations are susceptible to *B. parapertussis*, regardless of vaccination state.

B. pertussis and *B. parapertussis* infections present very similarly, with a biphasic display of symptoms. In the catarrhal stage, patients present with runny nose, low-grade fever, mild occasional cough and apnea (in infants). This stage lasts for 7 to 10 days. Both *B. pertussis* and *B. parapertussis* infections then progress to the paroxysmal stage, whereby patients present with classic pertussis symptoms including paroxysms followed by a high-pitched “whoop” and, in some cases, vomiting and cyanosis. The communicable stage occurs from disease onset to approximately 2 weeks-post paroxysmal cough stage (CDC—Clinical Features). Early and accurate diagnosis is critical for positive patient outcome as treatment is most effective within the first two weeks of infection. Previously vaccinated individuals may present with less severe disease and have an atypical clinical presentation.

The ARIES® *Bordetella* Assay uses Luminex Corporation’s real-time PCR chemistry in combination with the ARIES® Systems. The ARIES® Systems are capable of automated nucleic acid extraction and purification, real-time PCR detection of nucleic acid sequences, and data analysis. The ARIES® *Bordetella* Assay can directly detect and differentiate two respiratory pathogens: *B. pertussis* and *B. parapertussis* from nucleic acid in nasopharyngeal swab (NPS) specimens from patients with signs and symptoms of whooping cough. The ARIES® *Bordetella* Assay targets the pertussis toxin (ptxA) promoter and IS1001 repeat sequence in the genomes of *Bordetella pertussis* (*B. pertussis*) and *Bordetella parapertussis* (*B. parapertussis*), respectively. The promoter toxin gene targeted by the ARIES® *Bordetella* Assay confers a

high degree of specificity and is designed to detect only *B. pertussis* unlike other PCR-based assays that target the IS481 region, a multi-copy insertion element, which is present in multiple *Bordetella* species, including *B. pertussis*, *B. holmesii*, and *B. bronchiseptica* (Glare, et al., 1990; Reischl, et al., 2001 and Woolfrey and Moody 1991).

Principles of the Procedure

Each nasopharyngeal swab sample is collected from a patient using a commercially available nasopharyngeal swab (i.e., Flocked, Rayon, Polyester.) and then placed into an approved transport media (for example, Copan® Universal Transport Media (UTM™), ESwab™, M5®, M6™, or equivalent). The sample is then transported to the laboratory for testing. The sample is vortexed, then added to the sample chamber of an ARIES® *Bordetella* Assay cassette.

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® *Bordetella* Assay cassette is automatically scanned by the ARIES® instrument, associating a preloaded ARIES® *Bordetella* Assay protocol file with the cassette. The ARIES® *Bordetella* 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 *Bordetella* cassette. Purified nucleic acids are automatically transferred to the cassette's PCR tube that contains the lyophilized *Bordetella* Master Mix for the PCR amplification step. The *Bordetella* Master Mix contains primer pairs specific to *B. pertussis* and *B. parapertussis* and the SPC sequence. Total assay time, including extraction and PCR cycling, takes approximately two hours.

Materials Provided

The ARIES® *Bordetella* Assay (Part Number 50-10037) contains 24 assay cassettes.

The assay protocol file, package insert, and *ARIES® Quick Guide* ship separately on a USB as part of the ARIES® *Bordetella* Assay Protocol File Kit (CN-0388-01).

TABLE 1. **ARIES® *Bordetella* Assay Contents Provided By Luminex**

Item	Number	Description
ARIES® *Bordetella* Assay	50-10037	24 ARIES® *Bordetella* Assay cassettes which contain necessary reagents for sample extraction, nucleic acid purification, and amplification.
ARIES® *Bordetella* Assay Protocol File Kit	CN-0388-01	An assay protocol file, a package insert, and an *ARIES® Quick Guide* containing instructions for use are provided on a USB.

Materials Required But Not Provided

Reagents for sample collection:

- Nasopharyngeal swab (NPS)

Equipment:

- Appropriately sized pipettor
- Vortex mixer
- Luminex® ARIES® Systems (either an ARIES® System or an ARIES® M1 System can be used) and accessories
  - ARIES® magazines

- Sample Prep Tray
- Hand-held barcode reader

Plasticware and Consumables:

- Nuclease-free aerosol-barrier pipette tips

Warnings and Precautions

1. For *In Vitro* Diagnostic Use.
2. For prescription use only.
3. Handle all samples as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*, and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*.
4. Thoroughly clean and disinfect all surfaces with 10% bleach.
5. Avoid contamination from positive controls and samples by following good laboratory practices.
6. Avoid contamination by using a new nuclease-free aerosol barrier tip to add an individual sample aliquot to each cassette.
7. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash your hands thoroughly after performing the test.
8. Follow your institution's safety procedures for working with chemicals and handling biological samples.
9. Do not use cassettes, kits, or reagents beyond their expiration dates.
10. The cassettes are single-use. Do not reuse cassettes.
11. Store cassettes at the temperatures recommended on the cassette label. Do not freeze.
12. Only use the extraction protocol file provided by Luminex on the USB drive.
13. Only use the procedures described in this package insert. Any deviation from the outlined procedures can result in assay failure or cause erroneous results.
14. Only use ARIES® Systems that have been properly maintained according to the manufacturer's recommendations.
15. ARIES® cassettes contain guanidinium thiocyanate. Refer to the Safety Data Sheet (SDS) regarding safe handling practices for any spills.
16. In the event that a PCR tube falls off the cassette or a cassette leaks inside the ARIES® instrument, you should perform appropriate decontamination procedures to reduce the risk of contamination. Immediately clean all surfaces of the ARIES® magazine and the surrounding bench top with water. Wipe the surfaces with a lint-free cloth. Follow that with a fresh 10% bleach solution. Allow the bleach solution to sit for a minimum of 10 minutes. Thoroughly rinse bleached surfaces with deionized water. Dispose of all lint-free cloths in the appropriate waste container. Immediately contact Luminex Technical Support in order to retrieve the PCR tube from the ARIES® instrument. Do not throw away the cassette before you contact Technical Support. Do not attempt to retrieve the tube or put your hands inside the ARIES® instrument at any time. Do not proceed with additional testing until the PCR tube has been removed from the ARIES® instrument. Discard the cassette in accordance with the procedures defined by appropriate biohazard safety guidelines or regulations.
17. Refer to the appropriate ARIES® system operation manual for electrical and mechanical warnings.
18. Do not let the ARIES® Systems get wet or allow standing water to pool under the instrument.
19. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

Reagent Storage, Handling, and Stability

ARIES® *Bordetella* Assay cassettes are shipped refrigerated. Store at room temperature (15°C to 30°C) after receipt.

Always check the expiration date on the kit box and cassettes.

Sample Handling and Storage

Sample Collection

Nasopharyngeal swab (NPS) samples (i.e., Flocked, Rayon, Polyester) should be obtained by appropriately trained individuals and placed into an approved transport media (for example, Copan® Universal Transport Media (UTM™), ESwab™, M5®, M6™, or equivalent).

Sample Transport

When transporting biological samples, ensure that all applicable regulations for the transport of etiologic agents are met.

Transport samples refrigerated at 2°C to 8°C. If there will be a long delay before sample processing (greater than three days from the date of collection), samples should be frozen at -70°C or colder and transported on dry ice.

Sample Storage

Samples can be stored at 15°C to 30°C (room temperature) for up to 8 hours, or 2°C to 8°C (refrigerated) for up to 7 days. If testing with the ARIES® *Bordetella* Assay is not performed within 7 days of collection, then the sample must be frozen at ≤ -70°C for up to 5 months.

Assay Procedure

Adding Assay Files to the ARIES® Systems

The ARIES® *Bordetella* Assay protocol file is provided on the USB flash drive. The assay protocol file only needs to be imported to the ARIES® Systems once. To import the assay protocol file, complete the following:

1. Insert the USB flash drive into one of the five USB connectors (one in the front and four in the back).
2. Select  in the upper left-hand corner of the screen and navigate to **Assay Management**.
3. Select 

from the Page Action bar. The **Import File** dialog box displays.
4. Choose the **Location** and **File Name** of the assay file. Select **OK**.

Entering Orders

Sample barcodes are scanned to associate them with an order. An assay cassette is also then scanned to specify the assay and associate the cassette with a specific sample. Refer to *"Running an Assay"* on page 9 for more information.

The Sample ID is required on all orders and is the link between sample and cassette. The Accession ID and Requisition Number can also perform this role and associate the cassette to the sample, but are optional unless otherwise chosen to be required by the user. You can set requirement options in the Sample Options dialog box located on the Order Management Settings page.

Enabling the Automatic Print and Export Results Options

The Auto Print and Auto Export options are settings that need to be enabled prior to starting the run on the ARIES® instrument. Results can also be printed and exported manually after a run. Refer to *"Manually Printing Reports"* on page 10 for more information.

To enable the Auto Print feature, complete the following:

1. Select  in the upper left-hand corner of the screen and navigate to **Results > Settings**.
2. Toggle the **Generate Reports After Run** button to **Yes.**
3. For the **Sample Reports to Printer** option, select **Default** or **All**.

To export results automatically after a run, complete the following:

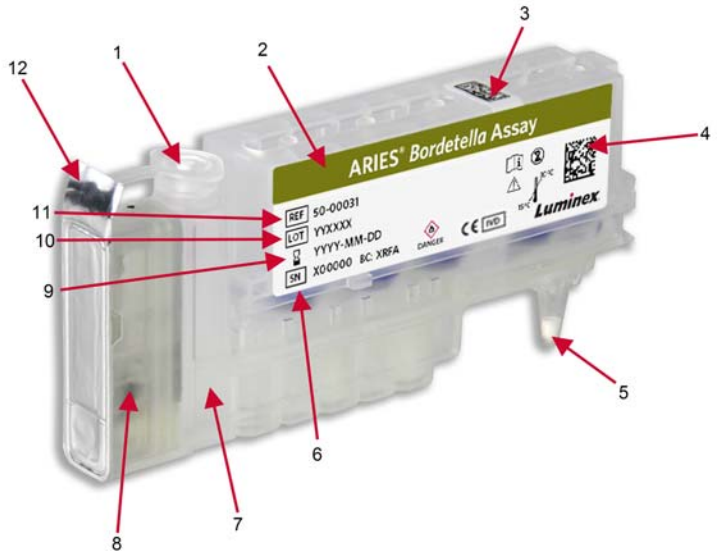
1. Select  in the upper left-hand corner of the screen and navigate to **Results > Settings**.
2. Toggle the **Summary Report as CSV** button to **Yes.**
3. Enter the **CSV Output Location** by selecting the folder icon in the upper right-hand corner of the **CSV Output Location** box. The **Select Folder** dialog box opens.
   a. Choose the **Location** for the export.
   b. Select **OK**.

To automatically export LIS results as either HL7 or CSV, complete the following:

1. Select  in the upper left-hand corner of the screen and navigate to **Administration > LIS Settings.**
2. Toggle the **Enable Export to LIS** button to **Yes**.
3. Toggle the **Auto Export to LIS** button to **Yes.**
4. Enter the **LIS Export Location** by selecting the folder icon in the upper right-hand corner of the **LIS Export Location** box. The **Select Folder** dialog box opens.
   a. Choose the **Location** for the export.
   b. Select **OK**.

   CAUTION: Validation of LIS compatibility must be performed by the user.

TABLE 2. **ARIES® Cassette**



1. Cassette cap	7. Sample chamber
2. Assay type	8. Side cassette
3. Cassette barcode (top)	9. Cassette expiration date
4. Cassette barcode (side)	10. Cassette lot number
5. PCR tube	11. Cassette part number
6. Cassette serial number	12. Back seal

Entering Orders on the ARIES® Systems

When entering orders, the Sample ID and Assay are required for an order to be valid.

NOTE: The order should be created prior to placing the cassette in the magazine. If you scan the cassette while the cassette is in the magazine, it is possible to scan the incorrect cassette barcode.

1. Select ☰ in the upper left-hand corner of the screen and navigate to **Order Management > Sample Orders**.
2. Select New Order from the Page Action bar. The **New Order** dialog box displays.
3. Remove the assay cassette from its packaging and visually inspect the cassette for any damage.

   CAUTION: If the cassette(s) or its packaging appears damaged in any way or if you see any leaks, DO NOT USE THE CASSETTE. Immediately contact Luminex Technical Support to report the damage.
4. Close the cassette cap to seal the cassette sample chamber.
5. Pick up and scan the barcode on the top (or side) of the cassette with the hand-held barcode reader or enter the required cassette information manually. A touch screen keyboard or a drop-down menu displays.

NOTE: If the keyboard does not automatically appear, toggle the keyboard icon to **Yes**. The keyboard will appear when you click in a field.

NOTE: If manually entering the **Cassette Lot Expiration**, select the calendar icon and choose the date using the calendar. The date is shown in the YYMMDD format.

a. If applicable, to add a control, choose **Control** in the **Sample Type** drop-down menu.
b. In the **Control** field, click the magnifying glass to select a control from the **Controls** dialog box.
c. Select the type of control in the **Control Type** drop-down menu.

NOTE: You can define the controls on the **Assay Management > Controls** page. Refer to the appropriate ARIES® system operation manual for more information on controls.

6. Pick up and scan the Sample ID on the sample tube or enter the required information manually.
7. Optionally, select the **Selected Tests** field. The **Test Selection** dialog box opens.
   a. Choose **Selected** or **Masked** for each test shown. Reports and results will not include data for any masked tests.

   NOTE: At least one test must remain unmasked. You cannot mask all tests.

   b. Select **Close**.
8. Scan the **Data Matrix** barcode on the screen next to **Save**, or manually select **Save**.

Adding Samples to the Cassettes

1. Place the sample tube in the Sample Prep Tray.
2. Pull the tab to remove the foil seal from the cassette.

CAUTION: Use caution when pulling the back seal off the cassettes. The foil is sharp and may cause injury.

3. Place the cassette in the Sample Prep Tray next to the sample.

4. Vortex the sample at high speed for 5 to 10 seconds to homogenize the mixture.
5. Open the cassette cap to access the cassette sample chamber.
6. Using an appropriately sized pipettor and aerosol barrier pipette tip, aspirate 200 µL of the sample from the sample tube.

CAUTION: Ensure that the correct amounts of sample are used.

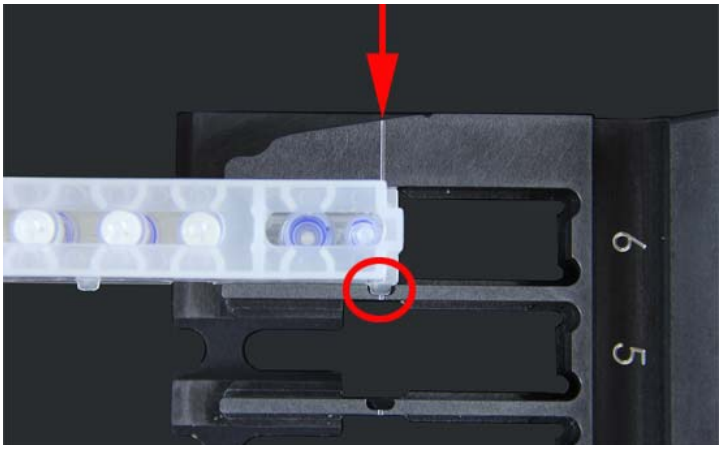
CAUTION: Use care to avoid contamination of the pipettor during transfer of the sample from the sample tube to the cassette.

7. Place the sample in the cassette sample chamber by inserting the pipette tip near the bottom of the chamber before expelling the sample.
8. Close the cassette cap to seal the cassette sample chamber.

CAUTION: Do not vortex or shake the cassette.

WARNING: Failure to ensure that the cassette cap is fully closed may cause a delay or failure in results and expose you to biohazards.

9. Place the cassette into the magazine by lining the cassette up with the first notch (a tab on the cassette fits into the notch).



NOTE: The PCR tube must face toward the numbers on the magazine.

10. Gently insert the cassette into the magazine.

11. Gently slide the cassette all the way back toward the numbers. Repeat for all other cassettes.

WARNING: Do not use your index finger to push the cassette into the magazine. You may indirectly dispense the reagent. Luminex recommends using the palm of your hand, or holding the cassette and sliding the cassette into the proper position.

Running an Assay

1. Select ☰ in the upper left-hand corner of the screen and navigate to **Run**.
   a. Insert the magazine into the ARIES® instrument. The ARIES® instrument automatically scans the barcode printed on the top of the ARIES® *Bordetella* Assay cassettes, and identifies associated orders and the proper assay protocol files before starting the run.

   NOTE: Ensure that the **Auto run upon Magazine Insertion** is toggled to **Yes** in the **Run Options** dialog box, located on the **Run > Settings** page. The instrument automatically scans the cassettes once the magazine is inserted and starts the run.

2. If there are any errors, the ARIES® instrument displays the specific error (for example, cassettes that cannot be run together, cassette IDs that have not been read, or assay files not loaded on to the ARIES® instrument). These errors must be corrected in order for the run to begin.
   If there are no errors, and the auto run option is selected, the color indicator of the cassette turns purple, the magazine state indicator indicates **PLEASE DO NOT REMOVE THE MAGAZINE** and an orange lock icon displays on the left-hand side of the magazine state indicator. The Run Status bar, located at the bottom of the **Run** page, displays an orange progress bar next to the estimated time to completion, colored purple. If you do not have the Auto Run feature enabled, you can start the run manually by selecting Start Run from the Page Action bar.

   NOTE: If you are using an ARIES® System with two modules, highlight the module you want before selecting **Start Run**.

Monitoring the Run

From the Run page, select Status on the Page Action bar to display the status of the magazine(s), the estimated time to completion, and the customizable name of the ARIES® instrument. This status screen is

intended to be visible from across the room, allowing you to monitor your runs while you are working on other projects.

TIP: On the Run > Settings page, you can customize whether the estimated completion time or estimated time remaining displays.

Reviewing, Printing, and Exporting Run Results

When the ARIES® *Bordetella* Assay run finishes successfully, the cassettes are colored green on the Run page. See *Table 3* for other color indicators. Refer to the appropriate ARIES® system operation manual for more color definitions.

TABLE 3. **Color Indicators**

Color	Reason
Red	Cassettes contain errors, were not scanned successfully, require additional information, or the run failed or was aborted. Contact Luminex Technical Support for assistance.
Yellow	Information was manually entered on the Run page or cassette is expired.
Green	Run finished successfully, the cassettes were scanned with no errors.
Blue	Magazine is inserted and a cassette is detected for this slot.
Purple	Module is currently running: the magazine slot is in use.
White	Empty module, no magazine is inserted or no cassette is detected.

The Run page includes visual indicators such as a status bar, an estimated time to completion indicator, and a Run Complete notification once the run has completed.

Automatically Printing and Exporting Results

NOTE: To ensure that the LIS Reporting (Auto Print) feature is enabled, check that Sample Reports to Printer is set to All or Default in the Export Settings dialog box located on the Results > Settings page.

When the run finishes successfully, the result reports are automatically printed at the default printer and exported in a CSV and PDF format to the designated location. Refer to *"Enabling the Automatic Print and Export Results Options"* on page 4.

Manually Printing Reports

To manually select a report to print, complete the following:

1. Select ☰ in the upper left-hand corner of the screen and navigate to **Results**. Regardless of the type of report you want to view, select only one result. Otherwise, the **Create Report** icon grays out.
2. Select **Create Report** from the Page Action bar. Choose the type of report you want to view from the drop-down menu. There are three options: **Run Report**, **Detailed Report**, and **Summary Report**.

   NOTE: Selecting a single result gives you the option to generate a **Run Report**, **Summary Report**, or a **Detailed Report**. You cannot select more than one result and run a **Summary Report** or a **Detailed Report** -- the **Create Report** icon grays out. When generating a **Run Report**, you can select multiple results from the same run and still use the **Create Report** icon. With **Run Report**, the **Create Report** icon is disabled only when

results from multiple runs are selected. Once the report opens, choose to export 

or print the result 

.

- The Run Report displays the run results for all samples in the run and any comments or logs associated with that run.
- The Summary Report displays the run result for one individual sample and any comments or logs associated with that sample.
- The Detailed Report displays all cassette information for one individual sample, the Amplification and Melt Graphs, and any comments or logs associated with that sample. Access to run this report is restricted to users with administrative rights.

Manually Exporting Results

To manually export results, complete the following:

1. Select the result(s) to export on the **Results** page and select 

from the Page Action bar.
2. Choose the **Location** and the **File Name** for the export in the **Export File** dialog box and select **OK**.

NOTE: You can only export to a USB drive or a mapped network drive.

Interpretation of Sample Results

The ARIES® software determines results for the sample and the sample processing control (SPC) based on the amplification cycle (Ct) value and the melting temperature (T_m) value provided in the assay protocol file. All assay outcomes are listed in *Table 4*.

TABLE 4. **Interpretation of Sample Results**

Example *	SPC		*B. pertussis*		*B. parapertussis*		Call
	T_m	Ct Value	T_m	Ct Value	T_m	Ct Value	
1	+	N/A	+	+	+	>	*B. pertussis* Positive *B. parapertussis* Negative
2	+	N/A	+	+	-	-	*B. pertussis* Positive *B. parapertussis* Negative
3	+	N/A	+	>	+	+	*B. pertussis* Negative *B. parapertussis* Positive
4	+	N/A	-	-	+	+	*B. pertussis* Negative *B. parapertussis* Positive
5	+	N/A	+	+	+	+	*B. pertussis* Positive *B. parapertussis* Positive
6	+	+	+	>	+	>	*B. pertussis* Negative *B. parapertussis* Negative
7	+	+	+	>	-	-	*B. pertussis* Negative *B. parapertussis* Negative
8	+	+	-	-	+	>	*B. pertussis* Negative *B. parapertussis* Negative
9	+	+	-	-	-	-	*B. pertussis* Negative *B. parapertussis* Negative

* All 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.

Legend	
+	Value meets acceptance criteria
-	Value does not meet acceptance criteria
N/A	Not applicable. All outcomes will result in the same call
>	Indicates that the Ct is beyond the Ct cutoff

Invalid Results

In case of an “Invalid” result, re-test the sample with a new assay cassette. If the problem is unresolved, contact Luminex Technical Support.

Quality Control

Quality control procedures intended to monitor the ARIES® Systems and assay performance are outlined in *Table 5*.

TABLE 5. **Control to Monitor Quality**

Control Type	Use
Sample Processing Control	Verifies proper sample lysis and nucleic acid extraction, and proper reagent, cassette, ARIES® instrument, and assay protocol performance.

Each ARIES® *Bordetella* 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, and federal accrediting organizations, as applicable.

Limitations

1. The detection of bacterial nucleic acids depends on proper sample collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to an incorrect result.
2. There is a risk of false negative results due to improperly collected, transported, or handled samples.
3. There is a risk of false negative results due to the presence of sequence variants in the targets of the assay, procedural errors, amplification inhibitors in samples, or inadequate numbers of organism(s) for amplification.
4. There is a risk of false positive results due to potential cross-contamination by target organism(s), their nucleic acid or amplified product, or from non-specific signals in the assay.
5. This test has been evaluated for use with human nasopharyngeal swab (NPS) specimen material only.
6. This test is a qualitative test and does not provide quantitative values of the detected organism.
7. This test has not been evaluated in patients without signs and symptoms of a respiratory tract infection.
8. The effect of interfering substances has been evaluated only for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
9. Cross-reactivity with respiratory tract organisms other than those tested can lead to erroneous results.
10. This test cannot rule out diseases caused by other bacterial or viral pathogens.
11. False negative results may occur due to the presence of strains with sequence variability or genetic rearrangements in the target regions of the ARIES® *Bordetella* Assay. For example, this was observed during analytical reactivity testing for the ARIES® *Bordetella* Assay with two low prevalence strains ATCC 8478 and ATCC 9797 (type strain 18323). Type strain 18323 is described in Bart et al. *mBio* 2014 5(2):e1074-14, with a *ptxA5* allele coding for the Ptx A subunit.
12. For use only on the ARIES® System or ARIES® M1 System.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Performance Characteristics

Clinical Performance

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 swab (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 assay 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 (Luminex Molecular Diagnostics, Toronto, ON).

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® *Bordetella* Assay 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 98.9% (1009/1020; LB 95% CI 98.1%) and 99.8% (1048/1050; LB 95% CI 99.3%), respectively.

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® *Bordetella* Assay results during 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 *Table 6* on page 15 and *Table 7* on page 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 6. **ARIES® *Bordetella* Assay Performance for *B. pertussis***

Specimen Description	PPA		95% CI	NPA		95% CI
Prospective	30/32*	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%

* 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 7. **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*	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%

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

Expected Results

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.

Analytical Performance

Limit of Detection

Limit of Detection (LoD) was established for the ARIES® *Bordetella* Assay using two strains each of *B. pertussis* and *B. parapertussis* diluted in natural negative nasopharyngeal (NP) swab matrix. The LoD is defined as the lowest sample concentration (CFU/mL) that had a positivity rate of ≥95%. Preliminary LoD concentrations were determined using serial dilutions of each *Bordetella* strain in natural negative nasopharyngeal (NP) swab matrix where each dilution was quantified independently. The preliminary LoD concentrations were then confirmed by testing 20 replicates of each strain.

The LoD for each *Bordetella* strain was determined and confirmed empirically as the lowest concentration that had ≥95% positive results. The LoD concentrations for each *Bordetella* strain are summarized in *Table 8*.

TABLE 8. **Limit of Detection of the ARIES® *Bordetella* Assay**

Assay Target	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%

Competitive Interference/Co-infection

Competitive interference was evaluated to confirm the ability of the ARIES® *Bordetella* Assay to detect *B. pertussis* or *B. parapertussis* in the presence of a co-infection. Competitive interference can occur when one organism is present in a specimen near the LoD and an additional organism that the assay is intended to detect is present at high concentration. *B. pertussis* and *B. parapertussis* combinations were tested in triplicate by mixing one target at a high concentration (≥10^6 CFU/mL) with the other target at a low concentration (3x LoD), and vice versa. All results were positive and in agreement with the expected results indicating the ARIES® *Bordetella* Assay can detect *B. pertussis* and *B. parapertussis* co-infections.

Interfering Substances

The potential inhibitory effect of non-microbial substances expected to be found in human nasopharyngeal swab specimens was evaluated by testing with the ARIES® *Bordetella* Assay. Three replicates each of *B. pertussis* and *B. parapertussis*, were tested at concentrations near the assay LoD with a clinically relevant concentration of each potentially interfering substance spiked into the reaction. All *B. pertussis* and *B. parapertussis* samples were 100% positive in the presence of a non-microbial substance at concentrations shown in *Table 9*; all negative samples containing only the non-microbial substance were 100% negative. The interfering substances used in the study are shown in *Table 9*.

TABLE 9. **Assay Interfering Substance Information**

Interfering Substance	Test Concentration
Benzocaine	2.5% (w/v)
Budesonide	25 mg/mL

Interfering Substance	Test Concentration
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)
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)

Analytical Specificity

Microbial interference for the ARIES® *Bordetella* Assay was assessed with 71 potential cross reactive microorganisms evaluated in the cross reactive study, and identified in *Table 10*. Each potential interfering microorganism was spiked into a natural negative human nasopharyngeal swab matrix containing a representative strain of *B. pertussis* or *B. parapertussis* near the LoD; bacteria were spiked and tested at concentrations ≥10^6 CFU/mL and viruses were spiked and tested at concentrations ≥10^5 $TCID_{50}$/mL, or the highest available concentration for both types of potential cross reactive microorganisms. All prepared samples were tested in triplicate (n=3) on the ARIES® instrument. *B. pertussis* was correctly detected in 3/3 replicates when tested in the presence of 66 cross reactive organisms (CROs), with 5 CROs *Bordetella bronchiseptica* (ATCC 19395 and ATCC 4617), *Bordetella petrii*, *Enterobacter aerogenes*, and *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.

TABLE 10. **Microorganism Information**

Microorganism			
1	*Acinetobacter baumanii*	38	Human Coronavirus OC43[3]
2	*Acinetobacter calcoaceticus*	39	Influenza A
3	*Acinetobacter Iwoffii*[9]	40	Influenza B[3]
4	Adenovirus 7A	41	*Klebsiella pneumoniae*[4]

Microorganism			
5	Adenovirus Type 1	42	*Lactobacillus acidophilus*
6	Adenovirus Type 3	43	*Lactobacillus plantarum*
7	*Arcanobacterium haemolyticum*[9]	44	*Legionella pneumophila*
8	*Bacteroides fragilis*	45	Metapneumovirus hMPV 20 Type A2
9	*Bordetella avium*	46	Metapneumovirus hMPV 8 Type B2
10	*Bordetella bronchiseptica (ATCC 19395)*[4, 5, 10]	47	*Moraxella catarrhalis*[3]
11	*Bordetella bronchiseptica (ATCC 4617)*[4, 6, 10]	48	*Morganella morganii*
12	*Bordetella bronchiseptica (ATCC BAA-588)*	49	Mumps virus
13	*Bordetella bronchiseptica (Clinical Isolate)*	50	*Mycobacterium tuberculosis*
14	*Bordetella hinzii*	51	*Mycoplasma pneumoniae*
15	*Bordetella holmesii (F061)*	52	*Neisseria elongata*
16	*Bordetella holmesii (C690)*	53	*Neisseria meningitidis*
17	*Bordetella holmesii (ATCC 51541)*[9]	54	*Oligella ureolytica*
18	*Bordetella holmesii (NCTC 13202)*	55	Parainfluenza Type 1
19	*Bordetella petrii*[4]	56	Parainfluenza Type 2
20	*Bordetella trematum*	57	Parainfluenza Type 3
21	*Burkholderia cepacia*	58	*Parvimonas micra*[1]
22	*Candida albicans*[7, 11]	59	*Proteus vulgaris*[3]
23	*Chlamydophila pneumoniae*	60	*Pseudomonas aeruginosa*[9]
24	*Citrobacter freundii*	61	*Ralstonia paucula*[2]
25	*Corynebacterium diptheriae*	62	Respiratory Syncytial Virus
26	Coxsackievirus[9]	63	Rhinovirus
27	Cytomegalovirus	64	*Staphyloccocus aureus*
28	Echovirus	65	*Staphyloccocus aureus (MRSA)*
29	*Enterobacter aerogenes*[4]	66	*Staphylococcus epidermidis*
30	*Enterococcus faecalis*	67	*Staphylococcus hominis*
31	Enterovirus	68	*Stenotrophomonas maltophilia*
32	Epstein-Barr virus	69	*Streptococcus pneumoniae*
33	*Escherichia coli*	70	*Streptococcus pyogenes*
34	*Fusobacterium necrophorum*[3]	71	*Streptococcus salivarius*
35	*Haemophilus influenzae*	72	*Bordetella pertussis*[8]
36	Human Bocavirus	73	*Bordetella parapertussis*[8]
37	Human Coronavirus 229E		

[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.

The analytical specificity of the ARIES® *Bordetella* Assay was evaluated by testing the potential cross reactivity of 71 microorganisms listed in *Table 10* on page 17. The microorganisms tested consisted of viral and bacterial strains representing common respiratory pathogens, or those potentially encountered in the human nasopharynx region. The potential cross-reacting organisms were spiked into natural nasopharyngeal matrix that was negative for *B. pertussis* and *B. parapertussis* and tested with the ARIES® *Bordetella* Assay in triplicate. Bacterial organisms were tested at concentrations ≥10^6 CFU/mL and viral organisms tested at ≥10^5 $TCID_{50}$/mL, or the highest available concentration for both types of potential cross reactive 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.

Carry-Over and Cross Contamination

Carry-over and cross contamination for the ARIES® *Bordetella* Assay were evaluated by testing 30 high concentration *B. pertussis* positive samples in a series alternating with 30 *B. pertussis* negative samples (natural negative human nasopharyngeal swab matrix). The high concentration positive samples were run adjacent to negative samples in five consecutive runs using one ARIES® instrument. No carry-over or cross contamination was observed, and the overall percent agreement with expected results was 100% for positive and negative samples.

Analytical Reactivity (Inclusivity)

The analytical reactivity/inclusivity of the ARIES® *Bordetella* Assay was evaluated with multiple strains of *B. pertussis* and *B. parapertussis*. A total of 18 unique strains were tested in triplicate at concentrations near the LoD. All reactivity strains for *B. pertussis* and *B. parapertussis* were detected with the exception of two *B. pertussis* strains, ATCC 8478 and ATCC 9797. Sequencing of these two strains showed that both strains contained a similar nucleotide mismatch in ARIES® *B. pertussis* primer binding regions, which presumably, impacts the ability of the ARIES® *Bordetella* Assay to detect these strains. An *in-silico* search of *B. pertussis* sequences in the NCBI database identified a low prevalence of strains with similar mismatches (2.9%). All mismatched strains identified had collection dates ≥10 years, with only one strain having a known human host—suggesting that these strains are not currently prevalent in the human population. The results of the inclusivity testing are shown in *Table 11*.

TABLE 11. **Analytical Reactivity for the ARIES® *Bordetella* Assay**

Organism	Inclusivity Strains	Lowest Concentration Tested[b]	ARIES® *Bordetella* Assay Result
B. pertussis	ATCC BAA-1335	5,400 CFU/mL	*B. pertussis* Detected
	ATCC 8467	5,400 CFU/mL	*B. pertussis* Detected
	ATCC 12742	5,400 CFU/mL	*B. pertussis* Detected
	ATCC 12743	5,400 CFU/mL	*B. pertussis* Detected
	ATCC 51445	5,400 CFU/mL	*B. pertussis* Detected
	E431	5,400 CFU/mL	*B. pertussis* Detected
	ATCC 10380	5,400 CFU/mL	*B. pertussis* Detected
	NR-42457	5,400 CFU/mL	*B. pertussis* Detected
	NR-42460	5,400 CFU/mL	*B. pertussis* Detected
	ATCC 8478	180,000 CFU/mL[a]	*B. pertussis* Not Detected
	ATCC 9797	180,000 CFU/mL[a]	*B. pertussis* Not Detected
B. parapertussis	ATCC 9305	639 CFU/mL	*B. parapertussis* Detected
	ATCC 15237	639 CFU/mL	*B. parapertussis* Detected
	ATCC 15311	639 CFU/mL	*B. parapertussis* Detected
	ATCC 15989	639 CFU/mL	*B. parapertussis* Detected
	C510	639 CFU/mL	*B. parapertussis* Detected
	E595	639 CFU/mL	*B. parapertussis* Detected
	E838	639 CFU/mL	*B. parapertussis* Detected

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

[b] *B. pertussis* and *B. parapertussis* strains were tested at 3x LoD.

Reproducibility (Site-to-Site)

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 reproducibility panel was prepared containing a moderate

positive (5x LoD) and low positive (1.5x LoD) independently for *B. pertussis* and *B. parapertussis* as well as a negative sample. The reproducibility panels were created by an independent operator and blinded to the testing sites. The result of the reproducibility study is shown in *Table 12*. Site-to-site Reproducibility Ct and Tm results are shown in *Table 13* on page 22. All dilutions were prepared in negative natural nasopharyngeal (NP) swab matrix; negative samples consisted of only negative natural NP matrix.

TABLE 12. **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%

* The expected result for Moderate Positive target was 100% Positive, Low Positive was ≥95% Positive, and Negative was 0% Positive.

TABLE 13. **Site-to-Site Reproducibility Ct and Tm Results for the ARIES® *Bordetella* Assay**

	Site 1				Site 2				Site 3				Overall Results			
Targets	**Avg Ct**	**Ct % CV**	**Avg T_m**	**T_m % CV**	**Avg Ct**	**Ct % CV**	**Avg T_m**	**T_m % CV**	**Avg Ct**	**Ct % CV**	**Avg T_m**	**T_m % CV**	**Avg Ct**	**Ct % CV**	**Avg T_m**	**T_m % CV**
***B. pertussis* (Low Positive)**	36.0	2.6%	81.4	0.07%	35.9	2.2%	81.3	0.11%	35.8	2.3%	81.4	0.08%	35.9	2.4%	81.4	0.09%
***B. pertussis* (Moderate Positive)**	34.3	1.7%	81.4	0.08%	34.1	2.1%	81.4	0.08%	34.2	1.2%	81.4	0.07%	34.2	1.7%	81.4	0.08%
***B. parapertussis* (Low Positive)**	34.4	2.1%	89.8	0.14%	33.8	1.9%	89.7	0.13%	34.2	1.5%	89.7	0.11%	34.2	2.0%	89.7	0.14%
***B. parapertussis* (Moderate Positive)**	32.4	1.4%	89.9	0.11%	32.0	1.9%	89.7	0.14%	32.4	1.4%	89.7	0.10%	32.3	1.7%	89.8	0.15%
Negative[a]	[a]31.5	5.6%	[a]81.2	0.43%	[a]31.9	5.5%	[a]81.2	0.45%	[a]32.8	6.7%	[a]81.3	0.18%	[a]32.1	6.1%	[a]81.2	0.37%

[a] Ct and T_m values for the *B. pertussis* and *B. parapertussis* Negative reflects DNA SPC values.

Precision

Within Laboratory Precision of the ARIES® *Bordetella* Assay was evaluated by two operators performing testing across two ARIES® Systems using a single lot of the ARIES® *Bordetella* Assay cassettes. Testing was performed on 5 nonconsecutive days and included a total of 150 replicates of a representative reproducibility panel. The reproducibility panel contains *B. pertussis* and *B. parapertussis* bacterial cultures diluted to two concentrations: moderate positive (5x LoD) and low positive (1.5x LoD). All dilutions were prepared in negative natural nasopharyngeal (NP) swab matrix; negative samples consisted of only negative natural NP swab matrix. See *Table 14*.

TABLE 14. **Within Laboratory Precision Results for the ARIES® *Bordetella* Assay**

Target Type	Expected Positivity	Positivity	95.0% 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%

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www.luminexcorp.com

Headquarters
Luminex, Austin

12212 Technology Blvd.
Austin, TX 78727
United States
Phone: +1-512-219-8020
North America Toll Free:
1-888-219-8020

Luminex, Madison

1224 Deming Way
Madison, WI 53717-1944
United States
Phone: +1-608-662-9000
North America Toll Free:
1-877-885-6617

Luminex, Toronto

439 University Avenue Suite 900
Toronto, Ontario
Canada M5G 1Y8
Phone: +1-416-593-4323
North America Toll Free:
1-800-593-2370

Luminex, Tokyo

Kamiyacho Sankei Bldg 3F 1-7-2 Azabudai
Minato-ku, Tokyo 106-0041
Japan
Phone: +81-3-5545-7440

Luminex, Shanghai

4/F C&D, Building 7, Pujiang Hi-Tech Park
2388 Chenhang Road, Minhang District
Shanghai 201114
China

Phone: +86-21-8036-9888

Luminex, The Netherlands

Het Zuiderkruis 1
5215 MV 's-Hertogenbosch
The Netherlands
Phone: +31-(0)73-800-1900