ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Package Insert

IVD For *In Vitro* Diagnostic Use.

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CE
EC REP

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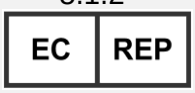
Document History Revision

Effective Date	Revision	Section	Description of Change
06/01/2022	A	ALL	Initial Release

Symbols Glossary

You will encounter these symbols throughout this package insert. They represent warnings, conditions, identifications, instructions, and regulatory agencies.

Symbol	Meaning	Symbol	Meaning
5.4.4[a] 	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[a] 	Manufacturer. Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
5.5.5[a] 	Contains Sufficient for <n> Tests. Indicates the total number of IVD tests that can be performed with the IVD kit reagents.	**BC**	Build Code
5.1.5[a] 	Batch Code. Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.4[a] 	Use-by date. Indicates the date after which the medical device is not to be used.
5.4.3[a] 	Consult instructions for use. Indicates the need for the user to consult the instructions for use.	5.1.6[a] 	Catalog(ue) Number. Indicates the manufacturer's catalogue number so that the medical device can be identified.
5.1.3[a] 	Date of manufacture. Indicates the date when the medical device was manufactured.	5.2.8[a] 	Do not use if package is damaged. Indicates a medical device that should not be used if the package has been damaged or opened.
5.1.2[a] 	Authorized representative in the European Community. Indicates the Authorized representative in the European Community	5.4.2[a] 	Do not re-use. Indicates a medical device that is intended for one use or for use on a single patient during a single procedure.

Symbol	Meaning	Symbol	Meaning
5.5.1[a] 	*In vitro* diagnostic medical device. Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.	5.4.1[a] 	Biological risks. Indicates that there are potential biological risks associated with the medical device.
GHS02[c] 	Danger. Highly flammable liquid and vapor.	5.1.7[a] 	Serial number. Indicates the manufacturer's serial number so that a specific medical device can be identified.
5.3.7[a] 	Temperature Limit. Indicates the temperature limits to which the medical device can be safely exposed.	[b] 	Conformite Europeenne (EU CE Marking of Conformity) CE conformity marking

a ANSI/AAMI/ISO 15223-1:2016, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

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

c ST/SG/AC.10/30/Rev.7 Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Seventh revised edition

Luminex Technical Support

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This manual can be updated periodically. For the latest version and related translations, contact Technical Support or visit https://www.luminexcorp.com/documents/.

Intended Use

The ARIES® Flu A/B & RSV+SARS-CoV-2 Assay is a Real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) based multiplexed nucleic acid test intended for use with the ARIES® Systems for the simultaneous qualitative detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals with clinical signs and symptoms of respiratory tract infections, including COVID-19.

The following organism types are identified using the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay:

Viral Target(s)
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
Influenza A
Influenza B
Respiratory Syncytial Virus (RSV)

Nucleic acids from the respiratory viral organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay may not be the definite cause of disease. Additional laboratory testing (*e.g.,* viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

Summary and Explanation of the Test

Respiratory Pathogens

Respiratory viruses are a leading cause of morbidity, hospitalization, and mortality worldwide. They cause acute local and systemic illnesses that range in severity and have the potential to cause severe disease especially in the young and elderly. Respiratory viruses are highly prevalent and are the most common cause of acute illness and physician visits in the U.S. (Tsukagoshi et al. 2013). The frequency of respiratory viral infections is highest in children under 4 years of age. School children get infected, on average, with 5 to 8 respiratory viruses per year, and adults average 2 to 4 respiratory viruses per year (Monto 1994; Turner 1998; Khabbaz et al. 2010).

Influenza Type A and B

Influenza is classified within the *Orthomyxoviridae* family of segmented, negative-strand enveloped RNA viruses (Cheng et al. 2012). Three influenza virus types (A, B, and C) have been identified, of which influenza A causes the most human infections. Influenza A is known to infect humans, pigs, birds, and horses, while influenza B primarily infects humans. Most human influenza epidemics and pandemics occur due to influenza A including the 2009 pandemic linked to the novel influenza A H1N1 strain, H1N1 pdm09. Influenza A is usually a more severe infection than influenza B, with influenza H3N2 strains have higher mortality. Avian influenza A subtypes, including H5N1, have resulted in human infection through contact with birds resulting in several outbreaks in Southeast Asia and the Middle East (Biggerstaff et al. 2014). Influenza viruses are generally transmitted by droplets with an incubation period of 1 to 4 days (La Rosa et al. 2013; Lessler et al. 2009). In North America, infection tends to occur in the winter months (Azziz Baumgartner et al. 2012). Seasonal epidemics of influenza can involve 10% or more of the entire population with a toll and cost much higher during a pandemic.

Respiratory Syncytial Virus (RSV)

Respiratory Syncytial Virus (RSV) is a member of the *Paramyxoviridae* family, and is a medium sized, enveloped virus with an antisense RNA genome (Chidgey and Broadley 2005). There are two subtypes of RSV, type A and type B. Illness caused by type A RSV may be more clinically severe than illness caused by type B. Transmission is via contact and through inhalation of droplets, with an incubation period of 3 to 7 days (La Rosa et al. 2013; Lessler et al. 2009). The incidence of RSV infections is seasonal, with outbreaks from November to April, peaking in December, January, and February (Chidgey and Broadley 2005; Simoes 2008). Globally, RSV is responsible for one third of the deadly childhood pneumonia cases (Meng et al. 2014).

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel viral respiratory pathogen that was first detected in Wuhan City, Hubei Province, China in 2019, and has been detected internationally, including cases in the United States (Ludwig, et al. 2020). The disease caused by the virus has been named “Coronavirus Disease 2019” (COVID-19), which has been designated a pandemic by the World Health Organization (WHO). SARS-CoV-2 is a member of the *Coronaviridae* family, which are small, enveloped virions with a positive-sense single-stranded RNA genome. SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption. The potential public health threat posed by COVID-19 is high, both globally and to the United States. To respond effectively to the COVID-19 outbreak, rapid detection of cases and contacts, appropriate clinical management and infection control, and implementation of community mitigation efforts are critical.

The ARIES® Flu A/B & RSV+SARS-CoV-2 Assay uses Luminex Corporation’s RT-PCR exonuclease chemistry in combination with the ARIES® Systems. The ARIES Systems are capable of automated nucleic acid extraction and purification, real-time RT-PCR detection of nucleic acid sequences, and data analysis. The ARIES Flu A/B & RSV+SARS-CoV-2 Assay can directly detect and differentiate four respiratory pathogens: influenza A virus (H1N1 (seasonal and pandemic 2009 (pdm09) and H3N2 (seasonal and variant (H3N2v)), influenza B virus (Yamagata and Victoria), respiratory syncytial virus (RSV-A and RSV-B), and SARS-CoV-2 from nucleic acid in nasopharyngeal swab (NPS) specimens from patients with signs and symptoms of respiratory tract infection.

Principles of the Procedure

The final format of the ARIES® Flu A/B & RSV+SARS-CoV-2 diagnostic assay uses a Luminex® RT-PCR chemistry in combination with the ARIES® cassette and instrument which is capable of automated nucleic acid extraction, purification, real-time RT-PCR detection of nucleic acid sequences, and data analysis. Purified nucleic acids are automatically transferred to the RT-PCR tube on the cassette that contains the lyophilized Flu A/B & RSV+SARS-CoV-2 Assay master mix for the RT-PCR amplification step. The lyophilized master mix contains primer/probe sets: one primer/probe set for the internal Sample Processing Control (SPC) target, and independent primer/probe sets each for the Flu A, Flu B, RSV and SARS-CoV-2 targets. In order to potentially reduce antigenic drift associated with SARS-CoV-2, two independent primer/probe sets were designed to two different genetic regions, E and N genes.

Primary nasopharyngeal swab (NPS) specimen in universal transport medium, Copan UTM® or BD™ UVT, is added directly to the ARIES Flu A/B & RSV+SARS-CoV-2 Assay cassette sample chamber. The cassette is then placed into an ARIES magazine which can hold up to six cassettes. The magazine is inserted into an ARIES instrument. A barcode on top of the ARIES Flu A/B & RSV+SARS-CoV-2 Assay cassette is automatically scanned by the ARIES instrument, associating a preloaded ARIES Flu A/B & RSV+SARS-CoV-2 Assay protocol file with the cassette. The ARIES Flu A/B & RSV+SARS-CoV-2 Assay protocol file contains the necessary parameters to run the cassette, analyze data, and generate reports. Total assay time, including extraction and PCR cycling, takes approximately two hours.

Assay Controls

- **Internal Sample Processing Control** – Sample Processing Control (SPC) primers and probe are included in the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay. In the absence of positive detection for SARS-CoV-2, influenza A, influenza B, and/or RSV target, positive detection for SPC verifies that nucleic acid is present in every sample. For negative samples, failure to detect SPC indicates a failure at either the extraction step, or the reverse-transcription step, or the PCR step.
- **Negative and Positive Controls** – Run a negative and positive control (not provided with the test kit) as good laboratory practice. Use external negative and positive controls in accordance with local, state, and federal accrediting organizations, as applicable.

Materials Provided

The ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Kit (Part number 50-10055) contains 24 assay cassettes.

The assay protocol file and a package insert ship separately on a USB as part of the ARIES Flu A/B & RSV+SARS-CoV-2 Assay Protocol File Kit (CN-0555-01) or request them from Luminex Technical Support.

Table 1: ARIES Flu A/B & RSV+SARS-CoV-2 Assay Contents Provided by Luminex

Item	Part Number	Description
ARIES Flu A/B & RSV+SARS-CoV-2 Assay Kit	50-10055	24 ARIES Flu A/B & RSV+SARS-CoV-2 Assay cassettes, which contain necessary reagents for sample extraction, nucleic acid purification, and amplification.
ARIES Flu A/B & RSV+SARS-CoV-2 Assay Protocol File Kit	CN-0555-01	A USB contains an assay protocol file and a package insert.

	Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Kit lots are identified on the kit carton label.

Materials Required but not Provided

Reagents for sample collection:

- Nasopharyngeal swab (NPS) (flocked tip or polyester swab)
- Universal Transport Medium (Copan UTM® or BD™ UVT)

Equipment:

- -65ºC to -95ºC freezer
- 2ºC to 8ºC refrigerator
- Luminex ARIES Systems (either an ARIES System or an ARIES M1 System can be used) and accessories
    - ARIES magazines
    - Sample Prep Tray
    - Handheld barcode reader
- Vortex mixer
- Appropriately sized pipettor

Plasticware and Consumables:

- Nuclease-free aerosol-barrier pipette tips

Reagent Storage, Handling, and Stability

ARIES cassettes are shipped at 2ºC to 30ºC. Store at room temperature (15ºC to 30ºC) after receipt.

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

Warnings and Precautions

1. For *In Vitro* Diagnostic Use.
2. For use by professionals trained to run the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay in a clinical laboratory.
3. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
4. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay protocol file is unique and will not run concurrently with any other ARIES Assay Cassettes in the same magazine.
5. Positive results are indicative of influenza A, influenza B, RSV and/or SARS-CoV-2 RNA.
6. Take care when handling, storing, and disposing of potentially infectious materials (i.e., samples, specimens, waste fluid, etc). Suitable barrier protection against potential pathogens is recommended during all stages of use. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with potentially infectious materials that may be unknown.
7. Handle waste disposal in accordance with accepted medical practice and applicable regulations. If spillage occurs, immediately disinfect following appropriate laboratory procedures.
8. Adhere to standard laboratory safety practices when handling hazardous, toxic, or flammable reagents and chemicals. Consult the package insert for the assay you are running and the Safety Data Sheet (SDS) for more information. Contact Luminex Technical Support when in doubt about compatibility of cleaning and decontamination agents or materials.
9. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Fresh clean gloves must be worn in each area and must be changed before leaving that area. Wash your hands thoroughly after performing the test.
10. Avoid contamination from positive controls and samples by following good laboratory practices.
11. Train personnel who use, maintain, or clean the instrument in standard laboratory safety practices and follow those practices when handling the instrument.
12. Follow your institution’s safety procedures for working with chemicals and handling biological samples.
13. Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases. Use only supplied or specifically required consumables to ensure optimal test performance.
14. Do not pipette by mouth.
15. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results.
16. Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Identify the lot number on the kit label.
17. Thoroughly clean and disinfect all surfaces with 10% household bleach.
18. Do not use cassettes, kits, or reagents beyond their expiration date.
19. The cassettes are single use. Do not reuse cassettes.
20. Store cassettes at the temperatures recommended on the cassette label. Do not freeze.
21. Only use the protocol file provided by Luminex on the USB drive.

22. Only use ARIES® Systems that have been properly maintained according to the manufacturer's recommendations.
23. ARIES cassettes contain guanidinium thiocyanate. Refer to the Safety Data Sheet (SDS) regarding safe handling practices for any spills.
24. 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. Followed with a fresh 10% household 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.
25. Do not let the ARIES Systems get wet or allow standing water to pool under the instrument.
26. Refer to the appropriate ARIES system operation manual for electrical warnings.
27. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
28. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

Sample Collection, Transport, and Storage

NOTE: Take standard precautions with regard to sample collection, handling, and storage prior to testing (refer to the latest edition of the CLSI MM13 Guideline; and Farkas et al. (1996)).

The recommended sample type for the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay is a nasopharyngeal swab (NPS) in Universal Transport Media (Copan UTM® or BD™ UVT) or equivalent transport media.

Samples can be stored at 15°C to 30°C for up to 3 hours, and 2°C to 8°C (refrigerated) for up to 72 hours. If testing with the ARIES Flu A/B & RSV+SARS-CoV-2 Assay is not performed within 72 hours of collection, then freeze the sample at -65°C to -95°C for up to 7 days.

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

Assay Procedure

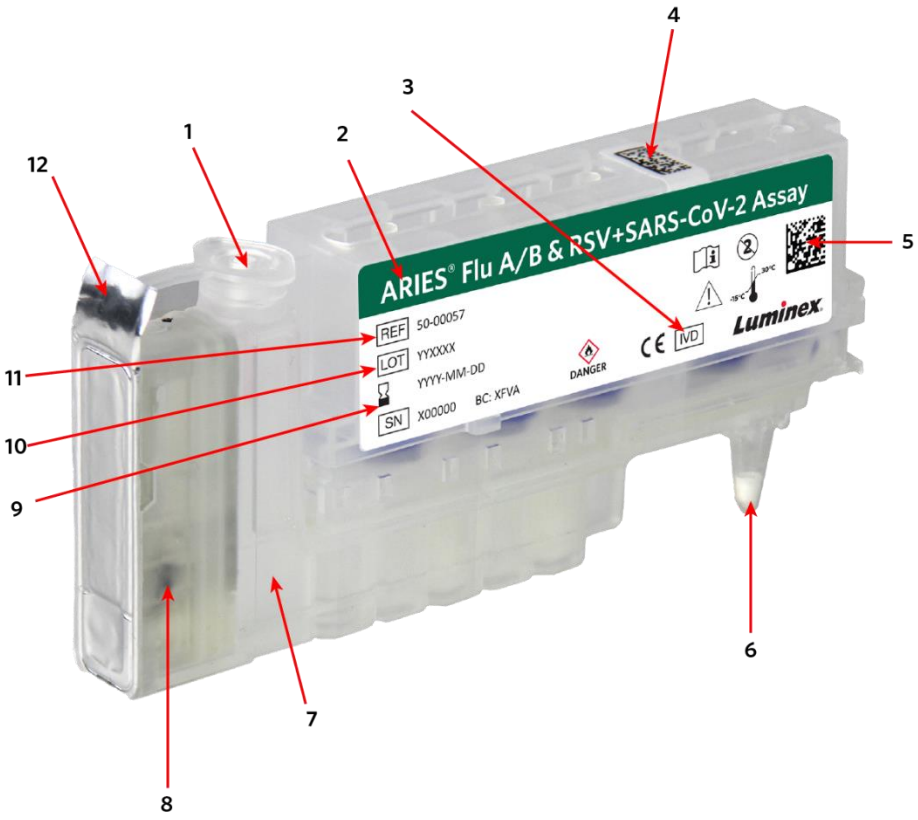
Software Setup

Import Assay Files to the ARIES Systems

The ARIES® Flu A/B & RSV+SARS-CoV-2 Assay protocol file is provided on the USB flash drive. Import the assay protocol file 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 **Import Assay** from the Page Action bar. The **Import File** dialog box displays.
4. Choose the **Location** and **File Name** of the assay file. Select **OK**.

   NOTE: The ARIES System can start runs automatically or manually. To ensure that the ARIES System will begin a run automatically, check that Auto run upon Magazine Insertion is toggled to Yes in the Run Options dialog box located on the Run > Settings page. For information on how to start runs manually, refer to the applicable ARIES system operation manual.



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

Add Samples to the Cassettes

⚠	Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Fresh clean gloves must be worn in each area and must be changed before leaving that area. Wash your hands thoroughly after performing the test.
	Avoid contamination from positive controls and samples by following good laboratory practices.

1. Place the sample tube in the Sample Prep Tray.
2. Remove the assay cassette from its packaging and visually inspect for any damage.

⚠	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.

3. Close the cassette cap to seal the cassette sample chamber.
4. Pull the tab to remove the foil seal from the cassette.

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

5. Place the cassette in the Sample Prep Tray next to the sample.
6. Vortex the sample for 5 to 10 seconds to homogenize the mixture.
7. Using an appropriately sized pipettor and aerosol barrier pipette tip, aspirate 200 µL of specimen.

8. Open the cassette cap and place the specimen in the cassette sample chamber by inserting the pipette tip near the bottom of the chamber before expelling the specimen.

⚠	Ensure the correct amount of sample is used.
	Use care to avoid contamination of the pipettor during transfer of the sample from the sample tube to the cassette.

9. Close the cassette cap to seal the cassette sample chamber.

⚠	Failure to ensure the cassette cap is fully closed may cause a delay or failure in results and expose you to biohazards.
	Do not vortex or shake the cassette.

Enter Orders on ARIES® Systems

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

NOTE: Create the order 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.

⚠	ARIES® Flu A/B & RSV+SARS-CoV-2 Assay protocol is unique and will not run concurrently with other ARIES Assay Cassettes in the same magazine.

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. 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.

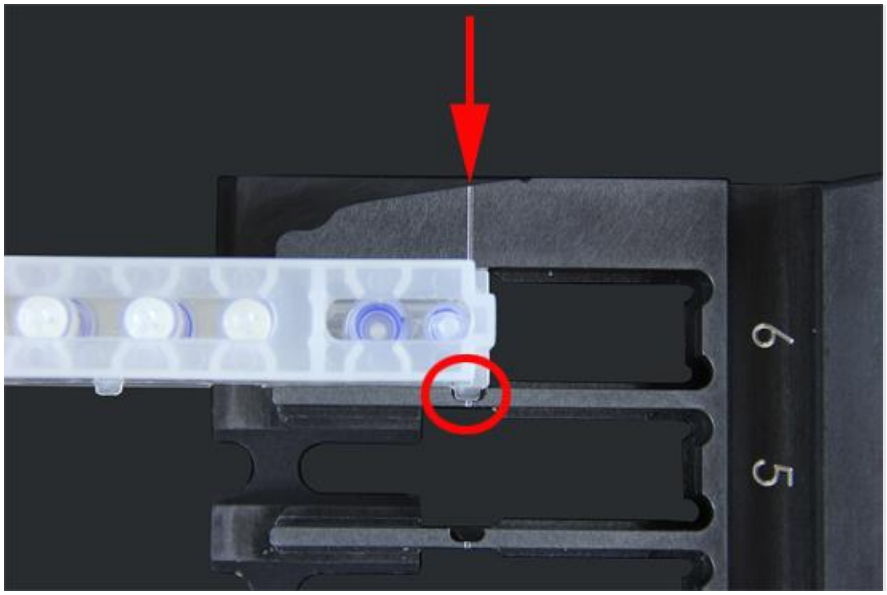
   NOTE: If the keyboard does not automatically appear, toggle the keyboard icon to Yes. The keyboard will appear when you click in a field.

   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 applicable ARIES® System operation manual for more information on controls.

5. Pick up and scan the Sample ID on the sample tube or enter the required information manually.
6. Scan the Data Matrix barcode on the screen next to **Save**, or manually select **Save**.
7. Place the cassette into the magazine by lining the cassette up with the first notch (a tab on the cassette fits into the notch).



8. Gently insert the cassette into the magazine.
9. Gently slide the cassette all the way back toward the numbers. Repeat for all other cassettes.

⚠	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.

Run the Assay

1. Select ☰ in the upper left-hand corner of the screen and navigate to **Run > Run**.
2. Insert the magazine into the ARIES® Instrument. The ARIES Instrument automatically scans the barcode printed on the top of the ARIES Flu A/B & RSV+SARS-CoV-2 Assay cassettes, 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.
3. 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.
   a. If **Auto run upon Magazine Insertion** is enabled and no errors occur, the instrument will automatically scan and start the run for you. The magazine status then indicates **PLEASE DO NOT REMOVE THE MAGAZINE** and an orange lock icon displays on the left-hand side of the magazine status.
   b. 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, 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.

Monitor 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.

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

Reports and Results

Refer to the applicable ARIES® system operation manual regarding reports and results.

Decontaminate the Sample Prep Tray

Luminex recommends cleaning the Sample Prep Tray (SPT) after each use to help avoid cross-contamination.

1. Wipe down the SPT surface with water and properly dispose of the cloth.
2. Wipe the SPT with 10% household bleach solution.
3. Rinse the SPT with water and allow to dry.

Cassette Disposal

Properly dispose of the cassettes.

	Handle waste disposal in accordance with accepted medical practice and applicable regulations. If spillage occurs immediately, disinfect following appropriate laboratory procedures. Take care when handling, storing, and disposing or potentially infectious materials (i.e., samples, specimens, waste fluid, etc.). Suitable barrier protection against potential pathogens is recommended during all stages of use. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with potentially infectious materials may be unknown. Adhere to standard laboratory safety practices when handling hazardous, toxic, or flammable reagents and chemicals. Consult the package insert for the assay you are running and the Safety Data Sheet (SDS) for more information. Contact Luminex Technical Support when in doubt about compatibility of cleaning and decontamination agents or materials.

Interpretation of Results

External controls are automatically analyzed and interpreted by the ARIES® software. If the external controls are not valid, do not interpret results until valid external control results are achieved.

The ARIES® Flu A/B & RSV+SARS-CoV-2 Assay detects targets using primers and probes specific to SARS-CoV-2, influenza A, influenza B, and Respiratory Syncytial Virus (RSV). Influenza A, influenza B, and RSV are each detected by a single, unique independent probe; SARS-CoV-2 is detected by two unique probes: one independent probe for detection of the E gene and one independent probe for detection of the N gene. Both probes utilize the same fluorophore resulting in a single SARS-CoV-2

result. The ARIES software determines results for the sample and the sample processing control (SPC) based on the amplification cycle (Ct) value and PCR signal amplification RFU (relative fluorescence units) provided in the assay protocol file. All assay outcomes are listed in Table 2.

Table 2. Interpretation of Sample Results

Influenza A	Influenza B	RSV	SARS-CoV-2	Sample Processing Control	Results Interpretation
+	+	+	+	+/-	Influenza A Positive, Influenza B Positive, RSV Positive SARS-CoV-2 Positive
-	+	+	+	+/-	Influenza A Negative, Influenza B Positive, RSV Positive SARS-CoV-2 Positive
+	-	+	+	+/-	Influenza A Positive, Influenza B Negative, RSV Positive SARS-CoV-2 Positive
+	+	-	+	+/-	Influenza A Positive, Influenza B Positive, RSV Negative SARS-CoV-2 Positive
+	+	+	-	+/-	Influenza A Positive, Influenza B Positive, RSV Positive SARS-CoV-2 Negative
-	-	+	+	+/-	Influenza A Negative, Influenza B Negative, RSV Positive SARS-CoV-2 Positive
-	+	-	+	+/-	Influenza A Negative, Influenza B Positive, RSV Negative SARS-CoV-2 Positive

Influenza A	Influenza B	RSV	SARS-CoV-2	Sample Processing Control	Results Interpretation
+	-	-	+	+/-	Influenza A Positive, Influenza B Negative, RSV Negative, SARS-CoV-2 Positive
-	+	+	-	+/-	Influenza A Negative, Influenza B Positive, RSV Positive, SARS-CoV-2 Negative
+	+	-	-	+/-	Influenza A Positive, Influenza B Positive, RSV Negative, SARS-CoV-2 Negative
+	-	+	-	+/-	Influenza A Positive, Influenza B Negative, RSV Positive, SARS-CoV-2 Negative
+	-	-	-	+/-	Influenza A Positive, Influenza B Negative, RSV Negative, SARS-CoV-2 Negative
-	+	-	-	+/-	Influenza A Negative, Influenza B Positive, RSV Negative SARS-CoV-2 Negative
-	-	+	-	+/-	Influenza A Negative, Influenza B Negative, RSV Positive, SARS-CoV-2 Negative
-	-	-	+	+/-	Influenza A Negative, Influenza B Negative, RSV Negative, SARS-CoV-2 Positive

Influenza A	Influenza B	RSV	SARS-CoV-2	Sample Processing Control	Results Interpretation
-	-	-	-	+	Influenza A Negative, Influenza B Negative, RSV Negative, SARS-CoV-2 Negative
-	-	-	-	-	Invalid

Luminex recommends that all co-infections, *i.e*., simultaneous infection by multiple pathogen species, be repeat tested with the same patient specimen, or if possible, with a newly collected specimen, to confirm test results.

Invalid Results

In case of an “Invalid” result, re-test beginning with the primary sample. Start at “Assay Procedure” and use a new assay cassette. If the problem is unresolved, contact Luminex Technical Support.

Quality Control

Quality control procedures intended to monitor ARIES® Systems and assay performance are outlined below.

Table 3. Controls to Monitor Quality

Control Type	Use
External Negative Control	Monitors for environmental contamination.
External Positive Control	Monitors the ARIES® System, cassettes, and assay protocols to ensure proper function.
Sample Processing Control	Verifies proper nucleic acid extraction, and proper reagent, cassette, ARIES instrument, and assay protocol performance.

Each ARIES® Flu A/B & RSV+SARS-CoV-2 Assay cassette contains a sample processing control, which is processed with the sample and analyzed during the amplification reaction. Test Positive and Negative control samples in accordance with appropriate federal, state, and local guidelines or accreditation requirements, as applicable.

Assay Limitations

1. This device may not be able to differentiate between newly emerging SARS-CoV-2 subtypes.
2. Based on *in silico* analysis of primer and probe sequences in the assay against sequences of analyzed organisms available in the GenBank Nucleotide database as of March 27, 2022, it is

predicted that the SARS-CoV-2 oligos are likely to detect some bat coronavirus and bat SARS-like coronavirus strains.

3. Performance of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay has only been established in nasopharyngeal swab (NPS) specimens.
4. Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
5. All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
6. The detection of pathogen nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
7. Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
8. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
9. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
10. There is a risk of false negative values due to the presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
11. A specimen yielding a negative result may contain respiratory pathogens not probed by the assay.
12. The performance of this assay was not established in immunocompromised patients.
13. The performance for some viruses and subtypes may vary depending on the prevalence and population tested.
14. The performance of this test has not been established for screening of blood or blood products.
15. This test cannot rule out infections caused by other viral or bacterial pathogens not present in this assay.
16. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
17. This device has been evaluated for use with human specimen material only.
18. The performance of this device has not been evaluated for patients without signs and symptoms of infection.
19. The performance of this device has not been evaluated for monitoring treatment of infection.
20. There is a risk of false negative results when a low concentration influenza A, influenza B, RSV, or SARS-CoV-2 pathogen is present in a specimen with a high concentration influenza A, influenza B, RSV, or SARS-CoV-2 pathogen, *e.g.*, dual infection.
21. 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.
22. Cross-reactivity with respiratory tract organisms other than those tested can lead to erroneous results.

23. For use only on the ARIES System or ARIES M1 System.

Performance Characteristics

Clinical Performance

Expected Values

ARIES® Flu A/B & RSV+SARS-CoV-2 Assay positive results (expected values) after allowable re-runs for each individual target are summarized for specimens included in the prospective study analysis per age group in Table 4 and per site in Table 5.

Table 4. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Expected Values for Prospective Specimens by Age Group (N=1023)

	0-1 year (N=208)		2-5 years (N=203)		6-21 years (N=225)		22-65 years (N=228)		> 65 years (N=158)		Overall (N=1023)	
	#	(%)	#	(%)	#	(%)	#	(%)	#	(%)	#	(%)
Influenza A	8	3.8% (8/208)	16	7.9% (16/203)	34	15.1% (34/225)	12	5.3% (12/228)	5	3.2% (5/158)	75	7.3% (75/1023)
Influenza B	0	0.0% (0/208)	0	0.0% (0/203)	0	0.0% (0/225)	0	0.0% (0/228)	0	0.0% (0/158)	0	0.0% (0/1023)
RSV	31	14.9% (31/208)	20	9.9% (20/203)	9	4.0% (9/225)	9	3.9% (9/228)	6	3.8% (6/158)	75	7.3% (75/1023)
SARS-CoV-2	22	10.6% (22/208)	17	8.4% (17/203)	24	10.7% (24/225)	70	30.7% (70/228)	16	10.1% (16/158)	149	14.6% (149/1023)

Table 5. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Expected Values for Prospective Specimens by Site (N=1023)

	Site 1		Site 2		Site 3		Site 4		Overall	
	#	(%)	#	(%)	#	(%)	#	(%)	#	(%)
Influenza A	13	3.2% (13/403)	54	9.7% (54/556)	1	3.1% (1/32)	7	21.9% (7/32)	75	7.3% (75/1023)
Influenza B	0	0.0% (0/403)	0	0.0% (0/556)	0	0.0% (0/32)	0	0.0% (0/32)	0	0.0% (0/1023)
RSV	40	9.9% (40/403)	33	5.9% (33/556)	1	3.1% (1/32)	1	3.1% (1/32)	75	7.3% (75/1023)
SARS-CoV-2	35	8.7% (35/403)	99	17.8% (99/556)	12	37.5% (12/32)	3	9.4% (3/32)	149	14.6% (149/1023)

The ARIES® Flu A/B & RSV+SARS-CoV-2 Assay reported multiple organism detections in a total of 9 prospective specimens. This represents 3.10% (9/290) of positive prospective specimens and 0.88% (9/1023) of all prospective specimens. All 9 coinfections contained 2 organisms. Out of the 9 specimens with multiple detections, 3 specimens (33%; 3/9) were concordant with the reference methods. Six (6) specimens (66%; 6/9) contained one or more organisms that were not detected by the reference/comparator methods (i.e., 6 false positive results).

The ARIES Flu A/B & RSV+SARS-CoV-2 expected values for all co-infection combinations are presented per age group in Table 6.

Table 6. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Co-infection Combinations for Prospective Specimens

	0-1 year (N=208)		2-5 years (N=203)		6-21 years (N=225)		22-65 years (N=228)		> 65 years (N=158)		Overall (N=1023)	
	#	(%)	#	(%)	#	(%)	#	(%)	#	(%)	#	(%)
Influenza A | RSV	0	0.0% (0/208)	1	0.5% (1/203)	0	0.0% (0/225)	0	0.0% (0/228)	0	0.0% (0/158)	1	0.1% (1/1023)
Influenza A | SARS-CoV-2	0	0.0% (0/208)	0	0.0% (0/203)	2	0.9% (2/225)	2	0.9% (2/228)	1	0.6% (1/158)	5	0.5% (5/1023)
RSV | SARS-CoV-2	2	1.0% (2/208)	1	0.5% (1/203)	0	0.0% (0/225)	0	0.0% (0/228)	0	0.0% (0/158)	3	0.3% (3/1023)

A multi-site clinical study established the performance of the ARIES Flu A/B & RSV+SARS-CoV-2 Assay for the detection and identification of influenza A (Flu A), influenza B (Flu B), respiratory syncytial virus (RSV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from nasopharyngeal swab (NPS) specimens collected from patients exhibiting clinical signs and symptoms of a respiratory tract infection (RTI). The clinical performance of the ARIES Flu A/B & RSV+SARS-CoV-2 Assay was evaluated using clinical specimens prospectively collected between October 2021 and March 2022 from four geographically diverse clinical sites within the United States. The clinical study utilized leftover, de-identified specimens collected from pediatric and adult patients exhibiting clinical signs and symptoms of RTI.

A total of 1145 unique prospective specimens were enrolled in the study, of which 1023 specimens were included in the prospective data analysis. Clinical runs and re-runs using the ARIES Flu A/B & RSV+SARS-CoV-2 Assay were tested on the ARIES® System by trained operators at four external clinical sites and one internal testing site. Due to the low prevalence of influenza B observed in the prospective study cohort, the prospective specimen set was supplemented with 123 pre-selected left-over, de-identified influenza B specimens (Arm 2) sourced from 2 sites in the United States. Pre-selected specimens were characterized by comparator method testing prior to enrollment in the study. To minimize bias, the pre-selected specimen set included negative specimens and was tested in a randomized, blinded manner at one internal site.

Out of the 1268 specimens enrolled in the prospective and pre-selected arms of the study, 122 (9.62%) specimens were disqualified and excluded from the study. Table 7 provides a summary of the general demographic information (age, gender, subject status) of the 1146 clinical specimens included in the prospective and pre-selected data analysis.

Table 7. General Demographic Details

	Prospective (N=1023)	Pre-selected (N=123)
	# Specimens (%)	# Specimens (%)
Gender		
Male	519 (50.7%)	64 (52.0%)
Female	504 (49.3%)	53 (43.1%)
Unknown	0 (0.0%)	6 (4.9%)
Sex Total	**1023 (100.0%)**	123 (100.0%)
Age (years)		
0-1	208 (20.3%)	10 (8.1%)
>1-5	203 (19.8%)	15 (12.2%)
>5-21	225 (22.0%)	20 (16.3%)
>21-65	228 (22.3%)	47 (38.2%)
>65	158 (15.4%)	6 (4.9%)
Unknown	1 (0.1%)	25 (20.3%)
Total	**1023 (100.0%)**	**123 (100.0%)**
Subject Status		
ER	489 (47.8%)	0 (0.0%)
Hospitalized	179 (17.5%)	0 (0.0%)
Long-Term Care	2 (0.2%)	0 (0.0%)
Outpatient	309 (30.2%)	0 (0.0%)
Unknown	44 (4.3%)	123 (100.0%)
Total	**1023 (100.0%)**	**123 (100.0%)**

The clinical performance of the ARIES Flu A/B & RSV+SARS-CoV-2 Assay was compared to a composite reference method testing algorithm including a molecular assay with confirmation of positive results by PCR followed by bi-directional sequencing (BDS) assays. For each target in the ARIES Flu A/B & RSV+SARS-CoV-2 Assay, the performance (Positive Percent Agreement, Negative Percent Agreement, and 95% confidence interval) of ARIES Flu A/B & RSV+SARS-CoV-2 Assay as compared to the composite reference method is summarized in Table 8 below for combined prospective and pre-selected specimen analysis.

Table 8. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Performance – Combined Prospective and Pre-selected

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Influenza A	Prospective	67/68	98.5%	92%-100%	947/955	99.2%	98%-100%
	Pre-Selected	0/0	N/A	N/A	123/123	100%	97%-100%
	Combined	**67/68[a]**	**98.5%**	**92%-100%**	**1070/1078[b]**	**99.3%**	**99%-100%**
Influenza B	Prospective	0/0	N/A	N/A	1023/1023	100%	100%-100%
	Pre-Selected	98/98	100%	96%-100%	25/25	100%	87%-100%
	Combined	**98/98**	**100%**	**96%-100%**	**1048/1048**	**100%**	**100%-100%**
RSV	Prospective	72/73	98.6%	93%-100%	947/950	99.7%	99%-100%
	Pre-Selected	0/0	N/A	N/A	123/123	100%	97%-100%
	Combined	**72/73[c]**	**98.6%**	**93%-100%**	**1070/1073[d]**	**99.7%**	**99%-100%**
SARS-CoV-2	Prospective	136/142	95.8%	91%-98%	868/881	98.5%	97%-99%
	Pre-Selected	0/0	N/A	N/A	120/123	97.6%	93%-99%
	Combined	**136/142[e]**	**95.8%**	**91%- 98%**	**988/1004[f]**	**98.4%**	**97% - 99%**

[a] The influenza A False Negative was negative by droplet digital PCR (ddPCR).
[b] All eight influenza A False Positives were negative by ddPCR. Four of the eight were positive for influenza A by Standard of Care (SoC) molecular assay.
[c] The RSV False Negative was positive for RSV by SoC molecular assay and ddPCR
[d] One of the three RSV False Positives was positive for RSV by SoC and ddPCR. The other two False Positives were negative by ddPCR.
[e] Of the six SARS-CoV-2 False Negatives, four were positive for SARS-CoV-2 by SoC, and four were positive by ddPCR.

[f] Of the 16 SARS-CoV-2 False Positives, two were positive for SARS-CoV-2 by SoC molecular assays. One SARS-CoV-2 False Positive was positive by ddPCR. Two pre-selected SARS-CoV-2 False Positives were negative for SARS-CoV-2 on repeat.

Out of the 1146 clinical specimens included in the prospective and pre-selected study analysis, 1139 (99.39%) generated valid ARIES Flu A/B & RSV+SARS-CoV-2 Assay results (i.e., Positive or Negative) on the first attempt. However, 3 of these 1139 specimens with valid results required retesting due to the detection of influenza A and influenza B coinfections (2) or run control failures (1). These 3 specimens combined with the 7 specimens with initial invalid results resulted in 10 specimens requiring retesting. All of the 10 specimens requiring retesting generated valid ARIES Flu A/B & RSV+SARS-CoV-2 Assay results after a single retest for a final success rate of 100% (1146/1146).

Analytical Performance

Limit of Detection (LoD)

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay using two representative strains for influenza A, influenza B, Respiratory Syncytial Virus (RSV), and Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The assay LoD was determined as the lowest concentration to achieve a positivity rate of ≥ 95% when the target was serially diluted in negative clinical matrix (NCM); NCM was a pool of human negative clinical specimens (NPS in UTM) that were pre-screened to be negative for influenza A, influenza B, RSV, and SARS-CoV-2. A preliminary LoD was established for each strain as the lowest concentration producing 100% positivity using a 5-point, 3-fold dilution series with three replicates at each dilution. The preliminary LoD was then confirmed by testing 20 replicates at concentrations at and below the LoD. The confirmed ARIES Flu A/B & RSV+SARS-CoV-2 Assay LoD concentrations for each strain tested are listed in Table 9.

Table 9: ARIES® Flu A/B & RSV + SARS-CoV-2 Assay Limit of Detection Results

Assay Target	Strain	LoD Concentration ($TCID_{50}$/mL or *PFU/mL)	LoD Concentration	Positivity
			(Copies/mL)	(n=20)
SARS-CoV-2	SARS-CoV-2 /Isolate: USA-WA 1/2020	2.35E+00	648	100%
	SARS-CoV-2/Hong Kong	2.20E-01	243	100%
Influenza A	A/Brisbane-02-2018/H1N1	5.77E+00	199	95%
	A/Kansas/14/2017 (H3N2)	3.17E+01	1472	100%
Influenza B	B/Colorado/06/2017	1.10E-01	375	100%
	B/Phuket/3073/2013	3.00E-01	627	100%
RSV	*RSV/A/Long	3.70E+01	832	100%
	RSV B WV/14617/85	5.40E+00	872	95%

Matrix Equivalency

The equivalency between the use of two matrices, pooled negative clinical matrix (NCM) and HeLa Negative Simulated Matrix (HL-NSM) on ARIES® Flu A/B & RSV+SARS-CoV-2 Assay was evaluated. Contrived samples of SARS-CoV-2, influenza A, influenza B, and RSV were prepared in both NCM and HL-NSM, using viral culture stocks at different test concentrations based on the Limit of Detection (LoD) results. NCM and HL-NSM were also tested as No Template Control (NTC) without spiking-in viral strains to confirm the absence of assay specific targets in both matrices. Equivalency for positive samples was demonstrated based on the positivity (%) results in target specific channels obtained in both NCM and HL-NSM. Equivalency for NTCs was demonstrated based on the positivity (%) results of sample processing control (AP559 channel). Results demonstrated equivalency between natural clinical matrix (NCM) and simulated matrix (HL-NSM) with the ARIES Flu A/B & RSV+SARS-CoV-2 Assay, as documented in Table 10.

Table 10: ARIES Flu A/B & RSV + SARS-CoV-2 Assay Matrix Equivalency Results

Assay Target	Strain	Testing Concentration		Sample Type	Matrix Type	
		($TCID_{50}$/mL or *PFU/mL)	(Copies/ mL)		Positivity (%) in HL-NSM	Positivity (%) in NCM
SARS-CoV-2	SARS-CoV-2 /Isolate: USA-WA 1/2020	1.17E+01	3240	5x LoD	100	100
		4.70E+00	1296	2x LoD	100	100
		5.86E-01	162	0.25x LoD	100	80
		2.35E-01	65	0.1x LoD	80	50
		NA	NA	NTC	100	100
	SARS-CoV-2 /HongKong/VM20001061/2020	1.09E+00	1215	5x LoD	100	100
		4.38E-01	486	2x LoD	100	98
		2.19E-02	23	0.1x LoD	50	10
		NA	NA	NTC	100	100
Influenza A	Flu A/Kansas/14/2017 (H3N2)	1.59E+02	7360	5x LoD	100	100
		6.35E+01	2944	2x LoD	100	100
		3.17E+00	147	0.1x LoD	40	20
		NA	NA	NTC	100	100
	Flu A/Brisbane/02/2018 (H1N1)	2.89E+01	995	5x LoD	100	100
		1.15E+01	398	2x LoD	98	98
		1.92E+00	66	0.3x LoD	90	20
		NA	NA	NTC	100	100
Influenza B	Flu B/Colorado/06/2017	5.44E-01	1874	5x LoD	100	100
		2.18E-01	750	2x LoD	100	100
		1.09E-02	37	0.1x LoD	50	10
		NA	NA	NTC	100	100
	Flu B/Phuket/3073/2013	1.49E+00	3134	5x LoD	100	100
		5.94E-01	1254	2x LoD	100	100
		2.97E-02	63	0.1x LoD	70	50
		NA	NA	NTC	100	100
RSV	*RSV A/ Long	1.85E+02	4160	5x LoD	100	100
		7.40E+01	1664	2x LoD	100	100
		3.70E+00	83	0.1x LoD	60	30
		NA	NA	NTC	100	100
	RSV B/ WV/14617/85	2.70E+01	4360	5x LoD	100	100
		1.08E+01	1744	2x LoD	100	100

Assay Target	Strain	Testing Concentration		Sample Type	Matrix Type	
		($TCID_{50}$/mL or *PFU/mL)	(Copies/ mL)		Positivity (%) in HL-NSM	Positivity (%) in NCM
		5.40E-01	87	0.1x LoD	30	40
		NA	NA	NTC	100	100

Positivity results of NTC samples recorded in Table 10 are based on the detection of sample processing control (AP559 channel).

Analytical Reactivity

The analytical reactivity (inclusivity) of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay was evaluated against 6 strains of SARS-CoV-2, 34 influenza A strains, 11 influenza B strains, and 6 RSV strains. These strains differ from those strains included in the Limit of Detection (LoD) study. Each strain was diluted in HeLa Negative Simulated Matrix (HL-NSM) to near LoD (3x LoD) testing concentration based on the confirmed LoD concentration for each analyte. Each reactivity strain was confirmed to be detected at a given concentration if 100% (3/3) of the replicates were positive. If a reactivity strain generated < 100% target positivity, that strain was prepared at a higher concentration and tested. All strains were detected at 3x LoD with the following exceptions: six influenza A strains (Influenza A/Indiana/02/2020 (H1N1 pdm09), Influenza A/Hong Kong/4801/14 (H3N2), Influenza A/Egypt/N03072/2010 (H5N1), Influenza A/Turkey/Virginia/4529/2002 (H7N2), Influenza A/Mallard/Netherlands/12/2000 (H7N7), and Influenza A/Hong Kong/33982/2009 (H9N2)) were detected at 30x LoD; and two influenza A strains (Influenza A/Hawaii/66/2019 X-345A (H1N1 pdm09) and Influenza A/CA/7/2009 NYMC x-179A (H1N1 variant)) were detected at 300x LoD. Three influenza B strains (Influenza B/Nevada/03/2011, Influenza B/Lee/40, and Influenza B/Panama/45/90 strains) were detected at 30x LoD. One RSV strain (RSVB/9320 strain) was detected at 30x LoD. The concentrations and measurement units at which the reactivity strains were detected on the ARIES Flu A/B & RSV+SARS-CoV-2 Assay are recorded in Table 11.

Table 11: ARIES® Flu A/B & RSV + SARS-CoV2 Assay Analytical Reactivity Results

Assay Target	Pathogen /Strain ID	Concentration Detected		LoD Concentration	Positivity (%)
SARS-CoV-2	SARS-CoV-2/Italy-INMI1/2020	7.05E+00	$TCID_{50}$/mL	3X	100
	SARS-CoV-2 (B.1.1.7 variant)/USA/CA_CDC_5574/2020	7.05E+00	$TCID_{50}$/mL	3X	100
	SARS-CoV-2 (B.1.351 variant)/South _Africa/KRISP-K005325/2020	7.05E+00	$TCID_{50}$/mL	3X	100
	SARS-CoV-2 (B.1.1.7 variant)/England/204820464/2020	7.05E+00	$TCID_{50}$/mL	3X	100
	SARS-CoV-2 (P1; gamma variant)/Japan/TY7-503/2021	7.05E+00	$TCID_{50}$/mL	3X	100

Assay Target	Pathogen /Strain ID	Concentration Detected		LoD Concentration	Positivity (%)
	SARS-CoV-2 (B.1.617.2; delta variant)/USA/PHC658/2021	7.05E+00	$TCID_{50}$/mL	3X	100
Influenza A	Influenza A/California/07/09 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Canada/6294/09 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Mexico/4108/09 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Michigan/45/15 (H1N1 pdm)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/New Caledonia/20/99 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Singapore/63/04 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Solomon Island/3/2006 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Swine/USA/1976/31 (H1N1)	1.73E+01	$CEID_{50}$/mL	3X	100
	Influenza A/Taiwan/42/06 (H1N1)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/WS/33 (H1N1)	1.73E+01	$CEID_{50}$/mL	3X	100
	Influenza A/NY/02/2009 (H1N1 pdm09)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/NY/03/2009 (H1N1 pdm09)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Indiana/02/2020 (H1N1 pdm09)	1.73E+01	$CEID_{50}$/mL	3X	33.3
	Influenza A/Indiana/02/2020 (H1N1 pdm09)	1.73E+02	$CEID_{50}$/mL	30X	100
	Influenza A/Guangdong-Maonan/SWL 1536/19 (H1N1 pdm)	1.73E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Hawaii/66/2019 X-345A (H1N1 pdm09)	1.73E+01	$CEID_{50}$/mL	3X	0

Assay Target	Pathogen /Strain ID	Concentration Detected		LoD Concentration	Positivity (%)
	Influenza A/Hawaii/66/2019 X-345A (H1N1 pdm09)	1.73E+02	$CEID_{50}$/mL	30X	0
	Influenza A/Hawaii/66/2019 X-345A (H1N1 pdm09)	1.73E+03	$CEID_{50}$/mL	300X	100
	Influenza A/CA/7/2009 NYMC x-179A (H1N1 variant)	1.73E+01	$CEID_{50}$/mL	3X	0
	Influenza A/CA/7/2009 NYMC x-179A (H1N1 variant)	1.73E+02	$CEID_{50}$/mL	30X	33.3
	Influenza A/CA/7/2009 NYMC x-179A (H1N1 variant)	1.73E+03	$CEID_{50}$/mL	300X	100
	Influenza A/California/7/04 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Hong Kong/4801/14 (H3N2)	9.52E+01	TCID50/mL	3X	0
	Influenza A/Hong Kong/4801/14 (H3N2)	9.52E+02	$TCID_{50}$/mL	30X	100
	Influenza A/Perth/16/09 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Port Chalmers/1/73 (H3N2)	9.52E+01	$CEID_{50}$/mL	3X	100
	Influenza A/Switzerland/9715293/13 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Texas/50/12 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Brisbane/10/07 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Victoria/361/2011 (H3N2)	9.52E+01	$CEID_{50}$/mL	3X	100
	Influenza A/Wisconsin/67/05 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Stockholm/6/14 (H3N2)	9.52E+01	$TCID_{50}$/mL	3X	100
	Influenza A/Indiana/8/2011 (H3N2 variant)	9.52E+01	$TCID_{50}$/mL	3X	100

Assay Target	Pathogen /Strain ID	Concentration Detected		LoD Concentration	Positivity (%)
	Influenza A/Egypt/N03072/2010 (H5N1)	5.97E+02	Copies/mL	3X	0
	Influenza A/Turkey/Virginia/4529/2002 (H7N2)	5.97E+02	Copies/mL	3X	33.3
	Influenza A/Mallard/Netherlands/12/2000 (H7N7)	5.97E+02	Copies/mL	3X	0
	Influenza A/Hong Kong/33982/2009 (H9N2)	5.97E+02	Copies/mL	3X	66.7
	Influenza A/Egypt/N03072/2010 (H5N1)	5.97E+03	Copies/mL	30X	100
	Influenza A/Turkey/Virginia/4529/2002 (H7N2)	5.97E+03	Copies/mL	30X	100
	Influenza A/Mallard/Netherlands/12/2000 (H7N7)	5.97E+03	Copies/mL	30X	100
	Influenza A/Hong Kong/33982/2009 (H9N2)	5.97E+03	Copies/mL	30X	100
	Influenza A/Anhui/1/2013 (H7N9)[a]	Diluted 100,000-fold	N/A	NA	100
	Genomic RNA from Kilbourne F38: A/Korea/426/1968 (HA, NA) x A/Puerto Rico/8/1934 (H2N2)[b]	Diluted 10,000-fold	N/A	NA	100
	Genomic RNA from Kilbourne F63: A/NWS/1934 (HA) x A/Rockefeller Institute/5/1957 (NA) (H1N2)[b]	Diluted 100,000-fold	N/A	NA	100
Influenza B	Influenza B/Alabama/2/17 (Victoria Lineage)	3.26E-01	$TCID_{50}$/mL	3X	100
	Influenza B/Brisbane/60/2008 (Victoria Lineage)	3.26E-01	$TCID_{50}$/mL	3X	100

Assay Target	Pathogen /Strain ID	Concentration Detected		LoD Concentration	Positivity (%)
	Influenza B/Florida/78/2015 (Victoria Lineage)	3.26E-01	$TCID_{50}$/mL	3X	100
	Influenza B/Nevada/03/2011 (Victoria Lineage)	3.26E-01	$CEID_{50}$/mL	3X	66.7
	Influenza B/Nevada/03/2011 (Victoria Lineage)	3.26E+00	$CEID_{50}$/mL	30X	100
	Influenza B/Florida/07/04 (Yamagata Lineage)	8.91E-01	$TCID_{50}$/mL	3X	100
	Influenza B/Lee/40 (Yamagata Lineage)	8.91E-01	$TCID_{50}$/mL	3X	66.7
	Influenza B/Lee/40 (Yamagata Lineage)	8.91E+00	$TCID_{50}$/mL	30X	100
	Influenza B/Massachusetts/2/2012 (Yamagata Lineage)	8.91E-01	$TCID_{50}$/mL	3X	100
	Influenza B/Panama/45/90 (Yamagata Lineage)	8.91E-01	$TCID_{50}$/mL	3X	0
	Influenza B/Panama/45/90 (Yamagata Lineage)	8.91E+00	$TCID_{50}$/mL	30X	100
	Influenza B/Texas/6/2011 (Yamagata Lineage)	8.91E-01	$TCID_{50}$/mL	3X	100
	Influenza B/Utah/9/14 (Yamagata Lineage)	8.91E-01	$TCID_{50}$/mL	3X	100
	Influenza B/Wisconsin/1/2010 (Yamagata Lineage)	8.91E-01	$CEID_{50}$/mL	3X	100
RSV	RSV/A/A1998/3-2	1.11E+02	$TCID_{50}$/mL	3X	100
	RSV/A/A2	1.11E+02	PFU/mL	3X	100
	RSV/A/A2000/3-4	1.11E+02	$TCID_{50}$/mL	3X	100
	RSV/B/9320	1.62E+01	$TCID_{50}$/mL	3X	66.7
	RSV/B/9320	1.62E+02	$TCID_{50}$/mL	30X	100
	RSV/B/CH93(18)-18	1.62E+01	$TCID_{50}$/mL	3X	100

Assay Target	Pathogen /Strain ID	Concentration Detected		LoD Concentration	Positivity (%)
	RSV/B/Wash/18537/62	1.62E+01	PFU/mL	3X	100

[a] Testing material from IRR provided with no titer/units information. Dilution factors used based on preliminary testing of these strains using the ARIES Flu A/B & RSV+SARS-CoV-2 assay prior to executing this verification study.

[b] Genomic RNA from BEI provided with no titer/units information. Test samples were prepared using HL-NSM and lysis buffer in 1:1 ratio to protect RNA against RNase activity. Dilution factors used based on preliminary testing of these strains using the ARIES Flu A/B & RSV+SARS-CoV-2 assay prior to executing this verification study.

In Silico Reactivity Analysis

Based on *in silico* inclusivity analysis, it is predicted that the SARS-CoV-2 sequences available from GISAID as of April 3, 2022, including sequences from all defined variants of concern or interest, are ~100% detectable by the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay.

Influenza A and B inclusivity was assessed with sequences available from the GISAID database between January 1, 2017 and April 3, 2022. The assay oligos for influenza A and influenza B are predicted to have ~100% inclusivity against the analyzed sequences.

For RSV, *in silico* inclusivity analysis was performed with sequences available from the GenBank nt database as of April 2, 2022. Based on this analysis, the RSV assay oligos are predicted to detect ~100% of the analyzed sequences.

Cross-Reactivity

Forty-four potential cross-reacting organisms commonly present at respiratory specimen collection sites were spiked into a HeLa Negative Simulated Matrix (HL-NSM) and tested in triplicate with the ARIES Flu A/B & RSV+SARS-CoV-2 Assay. Bacteria were tested at ≥ 1E+06 CFU/mL, viruses at ≥ 1E+05 $TCID_{50}$/mL, and organisms in which the quantitation is expressed in copies/mL, CFU/mL, IFU/mL, CCU/mL, or nuclei/mL at ≥ 1E+06. Results, shown in Table 12, demonstrated no cross-reactivity with the ARIES Flu A/B & RSV+SARS-CoV-2 Assay for any of the potentially cross-reacting organisms tested, as no influenza A, influenza B, RSV, or SARS-CoV-2 target positivity was observed in any replicates tested.

Table 12. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Analytical Specificity: Cross Reactivity

Cross-Reactive Organism	Test Concentration	Concentration Units	Positivity (%)
Adenovirus Type 1	1.00E+05	$TCID_{50}$/mL	0
Bordetella pertussis	1.00E+06	CFU/mL	0
Candida albicans	1.00E+06	CFU/mL	0
Chlamydia pneumoniae	1.00E+06	IFU/mL	0
Corynebacterium diphtheriae	1.00E+06	CFU/mL	0

Cross-Reactive Organism	Test Concentration	Concentration Units	Positivity (%)
Cytomegalovirus (CMV)	1.00E+05	U/mL	0
Enterovirus	1.00E+05	$TCID_{50}$/mL	0
Epstein-Barr Virus (EBV)	1.00E+05	cp/mL	0
Escherichia coli	1.00E+06	CFU/mL	0
Haemophilus influenzae	1.00E+06	CFU/mL	0
Herpes simplex virus	1.00E+05	U/mL	0
Human coronavirus 229E_ATCC	1.00E+05	$TCID_{50}$/mL	0
Human coronavirus 229E_Zepto	1.00E+05	$TCID_{50}$/mL	0
Human coronavirus HKU1	1.00E+05	$TCID_{50}$/mL	0
Human coronavirus NL63	1.00E+05	$TCID_{50}$/mL	0
Human coronavirus OC43_ATCC	1.00E+05	$TCID_{50}$/mL	0
Human coronavirus OC43_Zepto	1.00E+05	$TCID_{50}$/mL	0
Human Metapneumovirus	1.00E+05	$TCID_{50}$/mL	0
Human parainfluenza virus Type 1	1.00E+05	$TCID_{50}$/mL	0
Human parainfluenza virus Type 2	1.00E+05	$TCID_{50}$/mL	0
Human parainfluenza virus Type 3	1.00E+05	$TCID_{50}$/mL	0
Human parainfluenza virus Type 4A	1.00E+05	$TCID_{50}$/mL	0
Human parainfluenza virus Type 4B	1.00E+05	$TCID_{50}$/mL	0
Lactobacillus acidophilus	1.00E+06	CFU/mL	0
Legionella longbeachae	1.00E+06	CFU/mL	0
Legionella pneumophila	1.00E+06	CFU/mL	0
Measles	1.00E+05	$TCID_{50}$/mL	0
MERS-coronavirus	-	-	0
Moraxella catarrhalis	1.00E+06	CFU/mL	0

Cross-Reactive Organism	Test Concentration	Concentration Units	Positivity (%)
Mumps	1.00E+05	U/mL	0
Mycobacterium tuberculosis	1.00E+06	CFU/mL	0
Mycoplasma pneumoniae	1.00E+06	CCU/mL	0
Neisseria elongata	1.00E+06	CFU/mL	0
Neisseria meningitidis	1.00E+06	CFU/mL	0
Pneumocystis carinii	1.00E+06	nuclei/mL	0
Pseudomonas aeruginosa	1.00E+06	CFU/mL	0
Rhinovirus	1.00E+05	$TCID_{50}$/mL	0
SARS-coronavirus	-	-	0
Staphylococcus aureus	1.00E+06	CFU/mL	0
Staphylococcus epidermidis	1.00E+06	CFU/mL	0
Streptococcus pneumoniae	1.00E+06	CFU/mL	0
Streptococcus pyogenes	1.00E+06	CFU/mL	0
Streptococcus salivarius	1.00E+06	CFU/mL	0
Pooled Negative Clinical Matrix	-	-	0

In Silico Cross-Reactivity Analysis

For *in silico* exclusivity assessment of the assay oligos against on-panel and off-panel organisms listed in Table 13 below, based on analysis of sequences available in the GenBank nt database as of March 27, 2022, it is predicted that the SARS-CoV-2 oligos are likely to detect some bat coronavirus and bat SARS-like coronavirus strains.

Table 13. Potential Cross-Reactive Organisms assessed in the *In Silico* Exclusivity Analysis

On-Panel Organisms	Off-Panel Organisms	
Influenza A	Adenovirus	*Bordetella pertussis*
Influenza B	Cytomegalovirus	*Candida albicans*
Respiratory syncytial virus A	Enterovirus	*Chlamydia pneumonia*
Respiratory syncytial virus B	Epstein Barr Virus	*Corynebacterium diphtheriae*

On-Panel Organisms	Off-Panel Organisms	
SARS-CoV-2	Herpes simplex virus	*Escherichia coli*
	Human coronavirus 229E	*Haemophilus influenza*
	Human coronavirus HKU1	*Lactobacillus acidophilus*
	Human coronavirus NL63	*Legionella longbeachae*
	Human coronavirus OC43	*Legionella pneumophila*
	Human metapneumovirus (hMPV)	*Moraxella catarrhalis*
	Measles	*Mycobacterium tuberculosis*
	MERS-coronavirus	*Mycoplasma pneumonia*
	Mumps	*Neisseria elongata*
	Parainfluenza virus 1	*Neisseria meningitidis*
	Parainfluenza virus 2	*Pneumocystis carinii*
	Parainfluenza virus 3	*Pneumocystis jirovecii* (PJP)
	Parainfluenza virus 4	*Pseudomonas aeruginosa*
	Rhinovirus	*Staphylococcus aureus*
	SARS-coronavirus	*Staphylococcus epidermidis*
		Streptococcus pneumonia
		Streptococcus pyogenes
		Streptococcus salivarius

Interference

Microbial Interference

Results from the *in silico* analysis showed 14 potentially cross-reactive organisms with greater than or equal to 80% homology to one of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay primers/probes: Adenovirus Type 1, Adenovirus Type 7, *Candida albicans*, Enterovirus, *Haemophilus influenzae*, Human coronavirus 229E, Human coronavirus HKU1, Human coronavirus NL63, Human coronavirus OC43, Human Metapneumovirus, *Legionella pneumophila*, *Pseudomonas aeruginosa*, Rhinovirus, and SARS-coronavirus. These potentially interfering microorganisms were the only organisms evaluated as part of the microbial interference testing, as there was no cross reactivity detected with any of the cross-reactive organisms tested on the ARIES Flu A/B & RSV+SARS-CoV-2 Assay. Individual targets

(influenza A, influenza B, RSV, or SARS-CoV-2) were spiked in HeLa Negative Simulated Matrix (HL-NSM) at a concentration of 3X LoD. Each potentially interfering microorganism was individually spiked into the prepared target/HL-NSM at ≥ 1E+06 CFU/mL for bacteria, ≥ 1E+05 $TCID_{50}$/mL for viruses, and at ≥ 1E+06 for organisms in which quantitation is expressed in copies/mL, CFU/mL, IFU/mL, CCU/mL, or nuclei/mL. Each combination was tested in triplicate for interference. The ARIES Flu A/B & RSV+SARS-CoV-2 Assay generated 100% positivity (3/3) for each target (influenza A, influenza B, RSV, or SARS-CoV-2) in the presence of the potentially interfering microorganisms, indicating no interference with the ARIES Flu A/B & RSV+SARS-CoV-2 Assay. The results for microbial interference testing are presented in Table 14.

Table 14. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Analytical Specificity: Microbial Interference

Target	Interfering Microorganism	Test Concentration	Concentration Units	Target Positivity (%)
Influenza A (3X LoD)	Adenovirus Type 1	1.00E+05	$TCID_{50}$/mL	100
	Adenovirus Type 7	1.00E+05	$TCID_{50}$/mL	100
	Candida albicans	1.00E+06	CFU/mL	100
	Enterovirus	1.00E+05	$TCID_{50}$/mL	100
	Haemophilus influenzae	1.00E+06	CFU/mL	100
	Human coronavirus 229E	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus HKU1	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus NL63	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus OC43	1.00E+05	$TCID_{50}$/mL	100
	Human Metapneumovirus	1.00E+05	$TCID_{50}$/mL	100
	Legionella pneumophila	1.00E+06	CFU/mL	100
	Pseudomonas aeruginosa	1.00E+06	CFU/mL	100
	Rhinovirus	1.00E+05	$TCID_{50}$/mL	100
	SARS-coronavirus	-	-	100

Target	Interfering Microorganism	Test Concentration	Concentration Units	Target Positivity (%)
Influenza B (3X LoD)	Adenovirus Type 1	1.00E+05	$TCID_{50}$/mL	100
	Adenovirus Type 7	1.00E+05	$TCID_{50}$/mL	100
	Candida albicans	1.00E+06	CFU/mL	100
	Enterovirus	1.00E+05	$TCID_{50}$/mL	100
	Haemophilus influenzae	1.00E+06	CFU/mL	100
	Human coronavirus 229E	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus HKU1	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus NL63	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus OC43	1.00E+05	$TCID_{50}$/mL	100
	Human Metapneumovirus	1.00E+05	$TCID_{50}$/mL	100
	Legionella pneumophila	1.00E+06	CFU/mL	100
	Pseudomonas aeruginosa	1.00E+06	CFU/mL	100
	Rhinovirus	1.00E+05	$TCID_{50}$/mL	100
	SARS-coronavirus	-	-	100
RSV (3X LoD)	Adenovirus Type 1	1.00E+05	$TCID_{50}$/mL	100
	Adenovirus Type 7	1.00E+05	$TCID_{50}$/mL	100
	Candida albicans	1.00E+06	CFU/mL	100
	Enterovirus	1.00E+05	$TCID_{50}$/mL	100
	Haemophilus influenzae	1.00E+06	CFU/mL	100
	Human coronavirus 229E	1.00E+05	$TCID_{50}$/mL	100

Target	Interfering Microorganism	Test Concentration	Concentration Units	Target Positivity (%)
	Human coronavirus HKU1	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus NL63	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus OC43	1.00E+05	$TCID_{50}$/mL	100
	Human Metapneumovirus	1.00E+05	$TCID_{50}$/mL	100
	Legionella pneumophila	1.00E+06	CFU/mL	100
	Pseudomonas aeruginosa	1.00E+06	CFU/mL	100
	Rhinovirus	1.00E+05	$TCID_{50}$/mL	100
	SARS-coronavirus	-	-	100
SARS-CoV-2 (3X LoD)	Adenovirus Type 1	1.00E+05	$TCID_{50}$/mL	100
	Adenovirus Type 7	1.00E+05	$TCID_{50}$/mL	100
	Candida albicans	1.00E+06	CFU/mL	100
	Enterovirus	1.00E+05	$TCID_{50}$/mL	100
	Haemophilus influenzae	1.00E+06	CFU/mL	100
	Human coronavirus 229E	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus HKU1	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus NL63	1.00E+05	$TCID_{50}$/mL	100
	Human coronavirus OC43	1.00E+05	$TCID_{50}$/mL	100
	Human Metapneumovirus	1.00E+05	$TCID_{50}$/mL	100
	Legionella pneumophila	1.00E+06	CFU/mL	100

Target	Interfering Microorganism	Test Concentration	Concentration Units	Target Positivity (%)
	Pseudomonas aeruginosa	1.00E+06	CFU/mL	100
	Rhinovirus	1.00E+05	$TCID_{50}$/mL	100
	SARS-coronavirus	-	-	100

Competitive Inhibition (Co-Infection)

Performance of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay in the presence of clinically relevant co-infections (competitive interference) was evaluated using contrived samples, in which a low concentration analyte (near LoD) was tested in the presence of an additional analyte at high concentration, excluding RSV A + RSV B due to the assay's inability to distinguish between RSV subtypes. Results of the co-infection study are summarized in Table 15.

Table 15: ARIES® Flu A/B & RSV+SARS-CoV2 Assay Co-infection Results

Low Target Analyte (3x LoD)	High Target Analyte	High Target Concentration ($CEID_{50}$/mL, $TCID_{50}$/mL, PFU/mL)	Low Target Positivity (%)	High Target Positivity (%)
SARS-CoV-2/Hong Kong	Flu A/CA/7/2009 NYMC x-179A	1.00E+06[a]	100	100
	Flu B/Panama/45/90 (Yamagata)	1.00E+06	67	100
	Flu B/Panama/45/90 (Yamagata)	1.00E+05	100	100
	RSV A/Long	1.00E+05[b]	0	100
	RSV A/Long	1.00E+04[b]	100	100
	RSV B/CH93(18)-18	1.00E+05	0	100
	RSV B/CH93(18)-18	1.00E+04	0	100
	RSV B/CH93(18)-18	1.00E+03	100	100
Flu A/Kansas/14/2017 (H3N2)	SARS-CoV-2/USA-WA 1/2020	1.00E+05	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+04	33	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+03	100	100

Low Target Analyte (3x LoD)	High Target Analyte	High Target Concentration ($CEID_{50}$/mL, $TCID_{50}$/mL, PFU/mL)	Low Target Positivity (%)	High Target Positivity (%)
	Flu B/Panama/45/90 (Yamagata)	1.00E+06	100	100
	RSV A/Long	1.00E+05[b]	0	100
	RSV A/Long	1.00E+04[b]	100	100
	RSV B/CH93(18)-18	1.00E+05	0	100
	RSV B/CH93(18)-18	1.00E+04	100	100
Flu B/Phuket/3073/2013	SARS-CoV-2/USA-WA 1/2020	1.00E+05	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+04	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+03	67	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+02	100	100
	Flu A/CA/7/2009 NYMC x-179A	1.00E+06[a]	100	100
	RSV A/Long	1.00E+05[b]	0	100
	RSV A/Long	1.00E+04[b]	100	100
	RSV B/CH93(18)-18	1.00E+05	67	100
	RSV B/CH93(18)-18	1.00E+04	100	100
RSV A/Long	SARS-CoV-2/USA-WA 1/2020	1.00E+05	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+04	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+03	67	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+02	100	100
	Flu A/CA/7/2009 NYMC x-179A	1.00E+06[a]	100	100
	Flu B/Panama/45/90 (Yamagata)	1.00E+06	100	100
RSV B/WV/14617/85	SARS-CoV-2/USA-WA 1/2020	1.00E+05	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+04	0	100
	SARS-CoV-2/USA-WA 1/2020	1.00E+03	33	100

Low Target Analyte (3x LoD)	High Target Analyte	High Target Concentration ($CEID_{50}$/mL, $TCID_{50}$/mL, PFU/mL)	Low Target Positivity (%)	High Target Positivity (%)
	SARS-CoV-2/USA-WA 1/2020	1.00E+02	100	100
	Flu A/CA/7/2009 NYMC x-179A	1.00E+06[a]	100	100
	Flu B/Panama/45/90 (Yamagata)	1.00E+05	33	100
	Flu B/Panama/45/90 (Yamagata)	1.00E+04	100	100

[a] Flu A/CA/7/2009 NYMC x-179A (PN: VR1884, ATCC) viral culture stock concentration units are in $CEID_{50}$/mL.

[b] RSV A/ Long (PN: VR26, ATCC) viral culture concentration units are in PFU/mL.

Interfering Substances

The potential inhibitory effect of non-microbial substances that might be found in human nasopharyngeal swab (NPS) specimens was evaluated for the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay. Samples contrived with target (influenza A, influenza B, RSV, or SARS-CoV-2) at a concentration of 3X the assay Limit of Detection (LoD) in negative clinical matrix (NCM), were tested in triplicate with each potential interfering substance listed in Table 16 spiked at the highest, clinically relevant concentration. Additionally, three sample replicates of negative clinical matrix (NCM) were spiked with each potential interfering substance at the identical clinically relevant concentrations and tested for assay interference. The results of the study demonstrate that for every potential interfering substance tested the ARIES Flu A/B & RSV+SARS-CoV-2 Assay generated 100% positivity for each 3X LoD target (influenza A, influenza B, RSV, and SARS-CoV-2) and 0% positivity in the absence of target, apart from FluMist®; FluMist is an intranasal vaccine containing a panel of live, attenuated influenza A and influenza B viruses. To obtain 100% positivity for RSV and SARS-CoV-2, FluMist had to be diluted to 0.005%, from the initial testing concentration of 0.5%; for the negative samples, the positivity for influenza A and influenza B for 0.005% FluMist remained 100%. Based on this study, none of the substances tested interfered with the detection capabilities of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay apart from FluMist.

Table 16. ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Potentially Interfering Substances

Interfering Substance	Test Concentration	Concentration Units
Afrin®	5	% v/v
Benzocaine	2.5	% w/v
FLONASE®	5	% v/v
FluMist®[a]	0.5	% v/v

Interfering Substance	Test Concentration	Concentration Units
	0.005	
Human whole blood	2	% v/v
Menthol	1.7	mg/mL
Mucin	2	mg/mL
Mupirocin	6.6	mg/mL
Oseltamivir phosphate	1	µM
Phenylephrine	0.5	% w/v
Saline Spray	5	% v/v
Tobramycin	4	µg/mL
Zanamivir	3.3	mg/mL
Zicam®	5	% v/v
ESwab™	97.5	% v/v
MTM	97.5	% v/v
Remel M4-RT®	97.5	% v/v
VTM	97.5	% v/v
Flocked swab	-	-
Polyester swab	-	-
Rayon swab	-	-

[a] For FluMist, to obtain 100% positivity for RSV and SARS-CoV-2, the FluMist had to be diluted down to 0.005%; influenza A and influenza B remained 100% positive for all FluMist test concentrations. The negative samples spiked with FluMist were 100% positive for influenza A and influenza B.

Reproducibility and Repeatability

Site-to-site, within-lab (operator-to-operator), and within-run repeatability of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay were evaluated by testing a blinded panel of samples. Site-to-site reproducibility was evaluated at 3 sites, 2 of which were external to Luminex. Each site used a unique set of equipment for the study to test ten identical sets of blinded, randomized samples. The sample set for site-to-site reproducibility and precision/within-lab repeatability consisted of positive samples

representing all targets detected by the ARIES Flu A/B & RSV+SARS-CoV-2 Assay and a negative sample.

For site-to-site reproducibility and within-lab repeatability, a sample set consisting of 9 samples were tested in 3 replicates by 2 operators at each of the 3 sites using a single cassette lot across a minimum of 10 days total. Each target positive sample was prepared using representative viral strains of SARS-CoV-2, influenza A, influenza B, and RSV at 3x LoD [Low Positive, (LP)] and 10x LoD [Moderate Positive (MP)]. All samples were contrived in HeLa Negative Simulated Matrix (HL-NSM) and HL-NSM alone was used as negative sample. The data generated by operators 1 and 2 at Site 1 (Luminex) were used to evaluate within-lab (operator-to-operator), within-run, between-run, and instrument-to-instrument repeatability.

The results for site-to-site reproducibility and within laboratory repeatability are summarized in Table 17 and 18, respectively. The results demonstrated the reproducibility (across three sites) and repeatability (between operators) of the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay with an overall 99.6% call agreement with the expected results.

Site-to-Site Reproducibility

Table 17: ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Site-to-Site Reproducibility Results

Assay Target	Strain ID	Concentration	Call Agreement with Expected Results				
			Site 1	Site 2	Site 3	Overall (All Sites)	
Negative	NA	NA	100%	100%	100%	100%	100%
SARS-CoV-2	SARS-CoV-2/Hong Kong/VM20001061/2020	LP (3x LoD)	100%	100%	100%	100%	100%
		MP (10x LoD)	100%	100%	100%	100%	
Influenza A	Flu A/Brisbane-02-2018/H1N1	LP (3x LoD)	96.7%	96.7%	100%	97.8%	98.9%
		MP (10x LoD)	100%	100%	100%	100%	
Influenza B	Flu B/Colorado/06/2017	LP (3x LoD)	100%	100%	100%	100%	100%
		MP (10x LoD)	100%	100%	100%	100%	
RSV	RSV B WV/14617/85	LP (3x LoD)	100%	100%	96.7%	98.8%	99.4%
		MP (10x LoD)	100%	100%	100%	100%	
Overall Agreement (all targets / target types)			99.6% (269/270)	99.6% (269/270)	99.6% (269/270)	99.6% (807/810)	

Operator-to-Operator Repeatability

Table 18: ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Within Laboratory (Operator-to-Operator) Precision/Repeatability Results

Assay Target	Strain ID	Concentration	Call Agreement with Expected Results			
			Operator 1	Operator 2	Overall (Two Operators)	
Negative	NA	NA	100%	100%	100%	100%
SARS-CoV-2	SARS-CoV-2/Hong Kong/VM20001061/2020	LP (3x LoD)	100%	100%	100%	100%
		MP (10x LoD)	100%	100%	100%	
Influenza A	Flu A/Brisbane-02-2018/H1N1	LP (3x LoD)	93.3%	100%	96.7%	98.3%
		MP (10x LoD)	100%	100%	100%	
Influenza B	Flu B/Colorado/06/2017	LP (3x LoD)	100%	100%	100%	100%
		MP (10x LoD)	100%	100%	100%	
RSV	RSV B WV/14617/85	LP (3x LoD)	100%	100%	100%	100%
		MP (10x LoD)	100%	100%	100%	
Overall Agreement (all targets / target types)			99.3% (134/135)	100% (135/135)	99.6% (269/270)	

Lot-to-Lot Reproducibility

A reproducibility study was performed to evaluate the overall lot-to-lot reproducibility for the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay. Lot-to-Lot reproducibility was assessed by one operator using 1 ARIES® System to evaluate the sample panel across three unique cassette lots over 12 test days. The sample panel for each of the 4 assay targets consisted of 27 samples prepared in HeLa Negative Simulated Matrix (HL-NSM): nine replicates of a moderate positive (MP, 10x LoD) sample, nine replicates of a low positive (LP, 3x LoD) sample, and nine replicates of a negative (HL-NSM) sample. The sample panel for each target was tested in rotation to allow for five non-consecutive days between tests. Lot-to-lot reproducibility testing generated 100% call agreement with the expected results for the three unique lots. The data, presented as percent agreement to the expected results is shown in Table 19.

Table 19: ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Lot-to-Lot Reproducibility Results

	Agreement with Expected Results				
Target	**Target Concentration**	**Cassette Lot**			**Overall**
		AB5261	**AB5259**	**AB5779**	
SARS-CoV-2/Hong Kong/VM20001061/2020	MP (10X LoD)	100%	100%	100%	100% (27/27)
	LP (3X LoD)	100%	100%	100%	100% (27/27)
	Negative	100%	100%	100%	100% (27/27)
	Overall	100% (27/27)	100% (27/27)	100% (27/27)	100% (81/81)
Flu A/Brisbane-02-2018/H1N1	MP (10X LoD)	100%	100%	100%	100% (27/27)
	LP (3X LoD)	100%	100%	100%	100% (27/27)
	Negative	100%	100%	100%	100% (27/27)
	Overall	100% (27/27)	100% (27/27)	100% (27/27)	100% (81/81)
Flu B Colorado/06/2017	MP (10X LoD)	100%	100%	100%	100% (27/27)
	LP (3X LoD)	100%	100%	100%	100% (27/27)
	Negative	100%	100%	100%	100% (27/27)
	Overall	100% (27/27)	100% (27/27)	100% (27/27)	100% (81/81)
RSV B WV/14617/85	MP (10X LoD)	100%	100%	100%	100% (27/27)
	LP (3X LoD)	100%	100%	100%	100% (27/27)
	Negative	100%	100%	100%	100% (27/27)
	Overall	100% (27/27)	100% (27/27)	100% (27/27)	100% (81/81)

Within-Run Repeatability

Within-run and between-run repeatability was assessed using the within-lab (operator-to-operator) data generated at Site 1 (Luminex, Austin) and the results are presented in Table 20 and 21, respectively.

The evaluation of within-run and between-run repeatability of the assay was demonstrated with an overall 99.6% call agreement with the expected results for both studies.

Table 20: ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Within-run Repeatability Results

Reproducibility Panel Member	Call Agreement with Expected Results (%)										
	Run #1	Run #2	Run #3	Run #4	Run #5	Run #6	Run #7	Run #8	Run #9	Run #10	Overall
Negative	100	100	100	100	100	100	100	100	100	100	100
SARS-CoV-2 LP (3x LoD)	100	100	100	100	100	100	100	100	100	100	100
SARS-CoV-2 MP (10x LoD)	100	100	100	100	100	100	100	100	100	100	100
Influenza A LP (3x LoD)	100	66.7	100	100	100	100	100	100	100	100	96.7
Influenza A MP (10x LoD)	100	100	100	100	100	100	100	100	100	100	100
Influenza B LP (3x LoD)	100	100	100	100	100	100	100	100	100	100	100
Influenza B MP (10x LoD)	100	100	100	100	100	100	100	100	100	100	100
RSV LP (3x LoD)	100	100	100	100	100	100	100	100	100	100	100
RSV MP(10x LoD)	100	100	100	100	100	100	100	100	100	100	100
Overall % Agreement (all targets / target types)											99.6 (269/270)

LP: Low Positive

MP: Moderate Positive

Between Run Repeatability

Table 21: ARIES® Flu A/B & RSV+SARS-CoV-2 Assay Between Run Repeatability Results

Assay Target	Strain ID	Concentration	Call Agreement with Expected Results	
Negative	NA	NA	100%	100%
SARS-CoV-2	SARS-CoV-2/Hong Kong/VM20001061/2020	LP (3x LoD)	100%	100%
		MP (10x LoD)	100%	
Influenza A	Flu A/Brisbane-02-2018/H1N1	LP (3x LoD)	96.7%	98.3%
		MP (10x LoD)	100%	
Influenza B	Flu B/Colorado/06/2017	LP (3x LoD)	100%	100%
		MP (10x LoD)	100%	
RSV	RSV B WV/14617/85	LP (3x LoD)	100%	100%
		MP (10x LoD)	100%	
Overall Agreement (all targets / target types)			99.6% (269/270)	

Sample Carryover/Cross-Contamination

Carry-over and cross contamination for the ARIES® Flu A/B & RSV+SARS-CoV-2 Assay was evaluated by using samples consisting of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) viral culture fluid diluted into HeLa Negative Simulated Matrix (HL-NSM) at a concentration of 1.00E+05 $TCID_{50}$/mL. Testing was performed using 30 high positive SARS-CoV-2 samples in a series alternating with 30 negative (HL-NSM) samples. The high positive samples were run adjacent to negative samples across 10 consecutive runs on an ARIES® System. No carry-over or cross contamination was observed.

References

1. Azziz Baumgartner, E., et al. “Seasonality, timing, and climate drivers of influenza activity worldwide.” J Infect Dis. 2012 Sep 15; 206(6):838-46.
2. Biggerstaff, M., et al., “Estimates of the reproduction number for seasonal, pandemic, and zoonotic influenza: a systematic review of the literature.” BMC Infect Dis. 2014 Sep 4; 14:480.
3. Cheng, V.C., et al., “Two years after pandemic influenza A/2009/H1N1: what have we learned?” Clin. Microbiol. Rev. 2012 Apr; 25(2):223-63.
4. Chidgey, S.M. and K.J. Broadley, “Respiratory syncytial virus infections: characteristics and treatment.” J Pharm Pharmacol, 2005. 57(11):1371-81.
5. CLSI MM13 - Collection, Transport, Preparation and Storage of Specimens for Molecular Methods.
6. Farkas DH, Kaul KL, Wiedbrauk DL, Liechle FL. (1996) Specimen collection and storage for diagnostic molecular pathology investigation. Arch. Pathol. Lab. Med. 120: 591-596.
7. Khabbaz, R.F., et al., “Emerging and Reemerging Infectious Disease Threats”, in “Principles and Practice of Infectious Diseases”, G.L. Mandell, J.E. Bennet, and R. Dolin, Editors. 2010, Churchill Livingstone Elsevier: Philadelphia. p. 200-219.
8. Ludwig S, Zarbock A. Coronaviruses and SARS-CoV-2: A Brief Overview. Anesth. Analg. 2020 Jul; 131(1):93-96. doi: 10.1213/ANE.0000000000004845. PMID: 32243297; PMCID: PMC7173023.
9. Meng, J., et al. “An overview of respiratory syncytial virus.” PLoS Pathog. 2014 Apr 24; 10(4):e1004016. doi: 10.1371/-journal.ppat.1004016. eCollection 2014.
10. Monto, A.S., “Studies of the community and family: acute respiratory illness and infection.” Epidemiol. Rev., 1994. 16(2):351-73.
11. Simoes, E.A., “RSV disease in the pediatric population: epidemiology, seasonal variability, and long-term outcomes.” Manag. Care, 2008. 17(11 Suppl. 12):3-6, discussion 18-9.
12. Tsukagoshi, H., et al. “Molecular epidemiology of respiratory viruses in virus-induced asthma.” Front Microbiol. 2013 Sep 12; 4:278.
13. Turner, R.B., “The common cold.” Pediatr. Ann., 1998. 27(12):790-5.

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