Luminex

Package Insert | IVD

ARIES® Group A Strep Assay

IVD For *In Vitro* Diagnostic Use.

For Use With ARIES® Systems.

Rx Only

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ARIES® Group A Strep Assay Package Insert

89-30000-00-577 Rev. B
Assay Kit Part Number: 50-10041
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CE

EC REP

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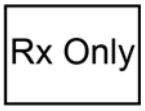
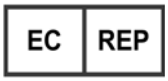
Luminex Corporation (Luminex) reserves the right to modify its products and services at any time. Notifications will be sent to end users regarding changes that impact the use, performance and /or safety and effectiveness of the device. Any modifications to the device will be made in accordance with 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.	5.3.7* 	Temperature Limit Indicates the temperature limits to which the medical device can be safely exposed.
5.1.5* 	Batch Code Indicates the manufacturer's batch code so that the batch or lot can be identified.	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.
5.1.6* 	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.
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
5.1.7* 	Serial Number Indicates the manufacturer's serial number so that a specific medical device can be identified.	% 	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)

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.	5.5.1* 	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
5.1.2* 	Authorized representative in the European Community Indicates the Authorized representative in the European Community	# 	Conformite Europeenne (EU CE Marking of Conformity) CE conformity marking

* 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

† ST/SG/AC.10/30/Rev.6 Globally Harmonized System of Classification and Labelling 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)

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Intended Use

The ARIES® Group A Strep Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*) in throat swab specimens from patients with signs and symptoms of pharyngitis.

The ARIES® Group A Strep Assay can be used as an aid in the diagnosis of Group A Streptococcal pharyngitis. The assay is not intended to monitor treatment for Group A *Streptococcus* infections.

The ARIES® Group A Strep Assay is indicated for use with ARIES® Systems.

Summary and Explanation of the Test

Streptococcus pyogenes (*S. pyogenes*), also referred to as Group A ß-hemolytic *streptococcus* (GAS), is a ubiquitous human pathogen that causes a broad spectrum of diseases. Millions of infections and over 500,000 deaths worldwide can be attributed to GAS each year (WHO 2005). *S. pyogenes* infection can occur in almost any human body tissue, however it most commonly infects the upper respiratory tract and skin lesions. (Efstratiou 2000)

Non-invasive ß-hemolytic Group A Streptococcal (GAS) pharyngitis is a significant cause of community-associated infections (Shulman *et al.* 2012). GAS is spread through person-to-person contact with infected individuals that may or may not be symptomatic. Up to 15% (Speert 1998) of school-aged children may carry the bacteria but are asymptomatic and are still capable of spreading the infection. Antibiotic therapy can be administered up to 9 days after onset of symptoms and still be effective in preventing GAS sequelae such as rheumatic fever (Shulman *et al.* 2012). An infected and ill individual that has been treated with antibiotics for twenty-four hours is unlikely to spread the infection.

Effective diagnosis of noninvasive GAS infections is critical for appropriate treatment. A diagnosis for GAS cannot be made based on the clinical features of pharyngitis alone since many viral agents such as rhinovirus or adenovirus often present similarly to a GAS infection (Bisno *et al.* 1997). Culture is the gold standard for diagnosis; it takes approximately two days to complete and has a sensitivity of 90% to 95% (Shulman *et al*. 2012). The current rapid strep tests have a sensitivity that ranges from 70% to 90% (false negative rate of 10% to 30%) (Gerber and Shulman 2004; Tanz *et al.* 2009). Negative tests are usually followed up with culture. Those patients who falsely test negative for GAS by rapid tests can be actively spreading disease while waiting for culture results. The goal of a molecular test for GAS pharyngitis is to improve the turn-around time and increase the sensitivity of detection compared with rapid antigen-based assays, thereby reducing the spread of GAS and minimizing inappropriate antibiotic use.

The ARIES® Group A Strep Assay uses Luminex Corporation's real-time PCR chemistry in combination with ARIES® Systems. ARIES® Systems are capable of automated nucleic acid extraction and purification, real-time PCR detection of nucleic acid sequences, and data analysis. The ARIES® Group A Strep Assay can directly detect *Streptococcus pyogenes* from nucleic acid isolated from throat swab specimens in Liquid Amies media from patients with signs and symptoms of pharyngitis.

Principles of the Procedure

Throat swab specimens are collected from patients using a commercially available Liquid Amies based transport system (Nylon Flocked Swab with 1 mL modified Liquid Amies (ESwab™)). The specimen is then transported to the laboratory for testing.

The primary sample is added directly to the ARIES® Group A Strep 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® Group A Strep Assay cassette is automatically scanned by the ARIES® instrument, associating a preloaded ARIES® Group A Strep Assay protocol file with the cassette. The ARIES® Group A Strep 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 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 ARIES® Systems and the ARIES® Group A Strep Assay cassette. Purified nucleic acids are automatically transferred to the cassette's PCR tube that contains the lyophilized Group A Strep Master Mix for the PCR amplification step. The lyophilized Group A Strep Master Mix contains two sets of primers: one primer set for *Streptococcus pyogenes*, and one for the SPC target. Total assay time, including extraction and PCR cycling, is approximately two hours.

Materials Provided

The ARIES® Group A Strep Assay (Part Number 50-10041) contains 24 assay cassettes.

The assay protocol file, package insert, and *ARIES® Quick Guide* ship separately on a USB as part of the ARIES® Group A Strep Assay Protocol File Kit (CN-0385-01).

TABLE 1. **ARIES® Group A Strep Assay Contents Provided By Luminex**

Item	Part Number	Description
ARIES® Group A Strep Assay	50-10041	24 ARIES® Group A Strep Assay cassettes which contain necessary reagents for sample extraction, nucleic acid purification, and amplification.
ARIES® Group A Strep Assay Protocol File Kit	CN-0385-01	An assay protocol file, a package insert, and *ARIES® Quick Guide* containing instructions for use, are provided on a USB.

Materials Required But Not Provided

Reagents for primary sample collection:

- Copan or BD™ Liquid Amies Elution Swab (ESwab™) Collection and Transport System (Nylon Flocked Swab with 1 mL Liquid Amies medium)

Equipment:

- 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

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. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
5. Thoroughly clean and disinfect all surfaces with 10% bleach.
6. Avoid contamination from positive controls and samples by following good laboratory practices.
7. Avoid contamination by using a new nuclease-free aerosol barrier tip to add an individual sample aliquot to each cassette.
8. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash hands thoroughly after performing the test.
9. Follow your institution's safety procedures for working with chemicals and handling biological samples.
10. Dispose of unused reagents and waste in accordance with local, county, provincial, state and federal regulations.
11. Do not use cassettes, kits, or reagents beyond their expiration date.
12. The cassettes are single-use. Do not reuse cassettes.
13. Store cassettes at the temperatures recommended on the cassette label. Do not freeze.
14. Only use the assay protocol provided by Luminex on the USB drive.
15. Only use the procedures described in this package insert. Any deviation from the outlined procedures can result in assay failure or cause erroneous results.
16. Only use ARIES® Systems that have been properly maintained according to the manufacturer's recommendations.
17. Test results should be interpreted in conjunction with other laboratory and clinical data.
18. Negative test results do not rule out other possible infections besides those caused by Group A *Streptococcus*.
19. Positive test results do not rule out co-infection with other pathogens.
20. ARIES® cassettes contain guanidinium thiocyanate. Refer to the Safety Data Sheets (SDS) regarding safe handling practices for any spills.
21. 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.
22. Refer to the appropriate ARIES® system operation manual for electrical and mechanical warnings.
23. Do not let the ARIES® Systems get wet or allow standing water to pool under the instrument.
24. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

Reagent Storage, Handling, and Stability

ARIES® Group A Strep 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

Throat swab samples should be obtained by appropriately trained individuals and stored in appropriate Liquid Amies Medium. Luminex recommends using a Nylon Flocked Swab for collection and stored in1 mL of Liquid Amies Medium (COPAN ESwab™ 480C or BD™ equivalent).

Sample Transport

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

Transport samples refrigerated at 4°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 20°C to 25°C (room temperature) for up to 48 hours or 4°C to 8°C (refrigerated) for up to seven days. If the sample cannot be tested within the indicated durations at the specified temperatures, then the sample can be stored at ≤-70°C for up to 6 months.

Software Setup

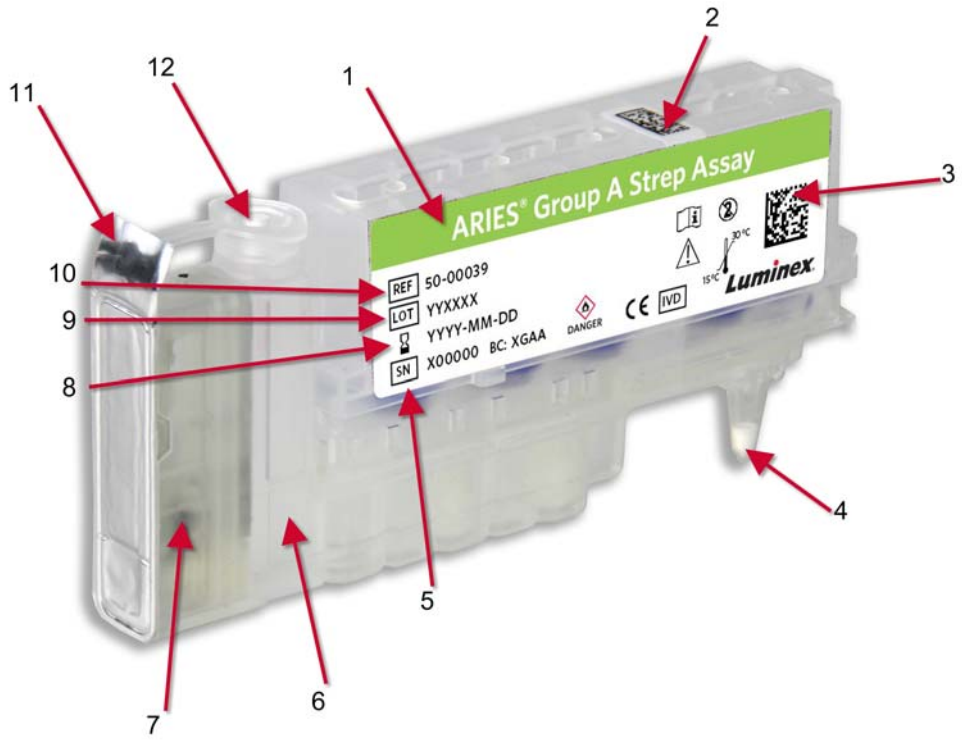
Importing Assay Files to ARIES® Systems

The ARIES® Group A Strep Assay protocol file is provided on the USB flash drive. The assay protocol file only needs to be imported to the ARIES® instrument 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**.

FIGURE 1. **ARIES® Cassette**



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

Assay Procedure

Adding Samples to the Cassettes

1. Remove the assay cassette from its packaging and visually inspect the cassette 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.

2. Close the cassette cap to seal the cassette sample chamber.
3. Place the sample tube in the Sample Prep Tray.
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 primary 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 sample from the sample tube.

Ensure that the correct amounts of sample are used.

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

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

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

Failure to ensure that 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.

10. Repeat steps 1-9 for remaining specimens to be tested.
11. Immediately proceed to order entry and initiation of the run on the ARIES® System.

If run initiation is delayed, prepare a new test cassette beginning from *Assay Procedure Step 1*, above.

Entering Orders on 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. 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.

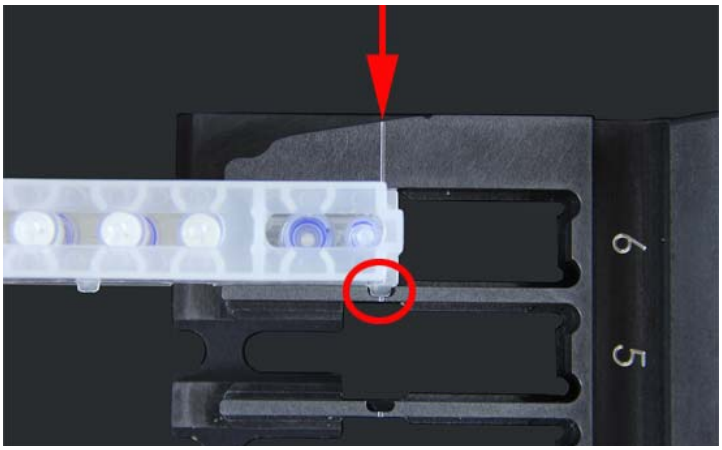
NOTE: If the keyboard does not automatically appear, toggle the keyboard icon to **Yes**. The keyboard will appear when you click in a field.

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.

4. Pick up and scan the Sample ID on the sample tube or enter the required information manually.
5. Scan the **Data Matrix** barcode on the screen next to **Save**, or manually select **Save**.
6. 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.

7. Gently insert the cassette into the magazine.
8. 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 and sliding the cassette into proper position.

Running an 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® Group A Strep 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.

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 state then indicates **PLEASE DO NOT REMOVE THE MAGAZINE** and an orange lock icon displays on the left-hand side of the magazine state. 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 

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 

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.

Reports and Results

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

Interpretation of Sample Results

The ARIES® software determines results for the sample and the sample processing control (SPC) for each sample 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 2*.

TABLE 2. **Interpretation of Sample Results**

Possible Outcomes	SPC		Group A *Streptococcus*		Interpretation
	Ct value	Tm	Ct value	Tm	
1	N/A	+	+	+	Group A *Streptococcus* Positive
2	+	+	>	+	Group A *Streptococcus* Negative
3	+	+	N/A	-	Group A *Streptococcus* Negative
4	-	+	N/A	-	Invalid
5	N/A	+	-	+	Invalid
6	N/A	-	N/A	N/A	Invalid

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 from the residual swab transport medium using a new assay cassette following the steps described in *“Adding Samples to the Cassettes”,* on page 5. If the problem is unresolved, contact Luminex Technical Support.

Quality Control

Quality control procedures intended to monitor ARIES® Systems and assay performance are outlined in *Table 3*.

TABLE 3. **Controls to Monitor Quality**

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

Each ARIES® Group A Strep Assay cassette contains a sample processing control, which is processed with the sample and analyzed during the amplification reaction.

External controls may be used in accordance with local, state, and/or federal regulations or accrediting organizations, as applicable. A reference strain of *Streptococcus pyogenes* (*S. pyogenes*) strain or well characterized *Streptococcus pyogenes* (*S. pyogenes*) clinical isolates may be used as a Positive Control. Liquid Amies medium may be used as a Negative Control. Alternatively, clinical specimens known to be positive or negative for *Streptococcus pyogenes* (*S. pyogenes*) may be used as Positive and Negative External Controls, respectively.

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 swab 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. False negative results may be obtained in the presence of NyQuil® (0.5% v/v).
5. False negative results may be observed in the presence of high concentrations of *Treponema denticola*.
6. The ARIES® Group A Strep Assay does not distinguish between viable and nonviable organisms and may produce a positive result in the absence of living organisms.
7. The ARIES® Group A Strep Assay does not differentiate asymptomatic carriers of Group A *Streptococcus* from those exhibiting streptococcal infection.
8. 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.
9. Additional follow-up testing by culture is required if the ARIES® Group A Strep Assay result is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF).
10. There is a risk of an “Invalid” result if a specimen contains Mucin proteins ≥ 5 mg/mL.
11. 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.

Expected Values

In a multi-center clinical study conducted in the U.S using patients presenting with pharyngitis, the prevalence of Group A *Streptococcus* was estimated to be 26.9% (166/618) using the ARIES® Group A Strep Assay and 25.9% (160/618) by culture.

Performance Characteristics

Clinical Performance

Performance of the ARIES® Group A Strep Assay was evaluated prospectively from January 2017 to May 2017 at four (4) geographically distinct clinical sites within the United States using the ARIES® System. Specimens included in the clinical study consisted of prospectively collected, de-identified, throat swab specimens from patients presenting with signs and symptoms consistent with pharyngitis. All eligible clinical specimens were tested by both reference method (bacterial culture followed by organism identification by Matrix-Assisted Laser Desorption/Ionization - Time-of-Flight Mass Spectrometry (MALDI-TOF MS)) and ARIES® Group A Strep Assay and the results compared. Reference method testing was performed at a centralized testing facility whilst ARIES® Group A Strep Assay testing was performed at each clinical site on their own clinical specimens.

A total of 735 throat swab specimens from subjects with signs and symptoms of pharyngitis were collected. Of these, 112 were excluded from the study due to failure to comply with the reference culture protocol or delay in reference culture (67), inclusion criteria not met or confirmed (30), insufficient specimen volume (7), use of an incorrect collection and transport device or eligibility not confirmed (3), lack of a pure reference isolate (2), testing performed by an ineligible operator (2) or prior enrollment of the subject (1), leaving a total of 623 unique specimens available for analysis. All 623 specimens were tested for Group A Strep by both the reference method and the ARIES® Group A Strep Assay. There were 6 specimens (6/623, 1.0%) that were re-tested with ARIES® Group A Strep Assay because they yielded initial invalid results due to run failure or instrument error. All six (6) specimens that were re-run generated valid ARIES® Group A Strep Assay results (i.e. positive or negative) after re-test. In addition, five (5) specimens generated inconclusive results by the comparator culture method (MALDI-TOF MS log (score) <2.00). All five (5) specimens with inconclusive reference results were excluded from the device performance calculations.

Clinical sensitivity of the ARIES® Group A Strep Assay for *S. pyogenes* against bacterial culture followed by identification with MALDI-TOF MS was 97.5% (156/160) with a lower bound 95% confidence interval of 93.7%. Clinical specificity of the ARIES® Group A Strep Assay for *S. pyogenes* against bacterial culture followed by identification with MALDI-TOF MS was 97.8% (448/458) with a lower bound 95% confidence interval of 96.0%. Positive Predictive Value (PPV) of the ARIES® Group A Strep Assay for *S. pyogenes* against bacterial culture followed by identification with MALDI-TOF MS was 94.0% (156/166) with a lower bound 95% confidence interval of 89.3%. Negative Predictive Value (NPV) of the ARIES® Group A Strep Assay for *S. pyogenes* against bacterial culture followed by identification with MALDI-TOF MS was 99.1% (448/452) with a lower bound 95% confidence interval of 97.7%.

TABLE 4. **ARIES® Group A Strep Assay Performance Compared to Bacterial Culture followed by identification with MALDI-TOF MS**

ARIES® Group A Strep Assay	**Bacterial Culture**		
	Positive	**Negative**	**TOTAL**
Positive	156	10[2]	166
Negative	4[1]	448	452
TOTAL	160	458	618[3]
		95% CI	
Sensitivity	97.5%	93.7% - 99.0%	
Specificity	97.8%	96.0% - 98.8%	
PPV	94.0%	89.3% - 96.7%	
NPV	99.1%	97.7% - 99.7%	

[1] Two (2) of the ARIES® Group A Strep Assay negative specimens that were positive by bacterial culture followed by identification with MALDI-TOF MS (i.e. False Negative) were Group A Strep negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Group A Strep Assay.

[2] Seven (7) of the ARIES® Group A Strep Assay positive specimens that were negative by bacterial culture followed by identification with MALDI-TOF MS (i.e. False Positive) were positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® Group A Strep Assay.

[3] Five (5) specimens generated inconclusive results by comparator culture method (MALDI-TOF MS log (score) <2.00). All five specimens were excluded from the device performance calculations.

Table 5 provides a summary of the general demographic information of the 623 prospectively collected throat swab specimens that were included in the prospective analysis.

TABLE 5. **General Demographic Details of the Clinical Study Population**

	Site 1 (N=105)	Site 2 (N=240)	Site 3 (N=130)	Site 5 (N=148)	Total (N=623)[1]
Gender					
Male	45 (42.9%)	115 (47.9%)	54 (41.5%)	71 (48.0%)	285 (45.7%)
Female	60 (57.1%)	125 (52.1%)	76 (58.5%)	77 (52.0%)	338 (54.3%)
Age (yrs)					
<=18 years	105 (100.0%)	240 (100.0%)	90 (69.2%)	148 (100.0%)	583 (93.6%)
> 18 years	0 (0.0%)	0 (0.0%)	40 (30.8%)	0 (0.0%)	40 (6.4%)
Subject Status					
Hospitalized	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (2.7%)	4 (0.6%)
Emergency Department	105 (100.0%)	0 (0.0%)	0 (0.0%)	144 (97.3%)	249 (40.0%)
Outpatient Clinic	0 (0.0%)	240 (100.0%)	130 (100.0%)	0 (0.0%)	370 (59.4%)
Immune Status					
Immuno-compromised	1 (1.0%)	0 (0.0%)	1 (0.8%)	2 (1.4%)	4 (0.6%)
Immuno-competent	104 (99.0%)	240 (100.0%)	129 (99.2%)	146 (98.6%)	619 (99.4%)

[1] Includes five (5) subjects whose culture results were inconclusive but who had valid ARIES® test results.

Analytical Performance

Limit of Detection

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES® Group A Strep Assay (GAS) using two strains of *Streptococcus pyogenes* (*S. pyogenes*) diluted in negative clinical matrix (NCM) (pool of Group A *Streptococcus* negative clinical specimens - throat swabs in Liquid Amies). The LoD for each strain was determined as the lowest concentration that had a positivity rate of ≥95%. Preliminary LoD concentrations were determined using serial dilutions of each strain. All *S. pyogenes* strain concentrations were determined by plating and colony counting (CFU/mL). The preliminary LoD concentrations were confirmed by testing twenty (20) replicates of each strain. The final LoD concentrations are shown in *Table 6*.

TABLE 6. **ARIES® Group A Strep Assay Limit of Detection Results**

Assay Target	Strain	LoD Concentration (CFU/mL)	Positivity	95% Confidence Interval
Streptococcus pyogenes	Bruno [CIP 104226] (ATCC® 19615™)	2.58E+03	19/20 (95%)	75.1% - 96.8%
Streptococcus pyogenes	SF370; M1 GAS (ATCC® 700294™)	4.13E+03	20/20 (100%)	83.2% - 100.0%

Interfering Substances

The potential interfering effect of non-microbial substances expected to be found in human throat swab specimens was evaluated for the ARIES® Group A Strep Assay by testing three replicates of GAS culture near the assay LoD spiked with the highest potential concentration of each substance. The expected results were obtained under all conditions except in the presence of NyQuil® and mucin. Two of three samples containing 0.5% (v/v) NyQuil produced false negative results on initial testing, although repeat analysis under the same condition produced the expected results. Invalid results were obtained in the presence of mucin at 5mg/mL although additional testing demonstrated no interference at concentrations ≤4mg/mL.

TABLE 7. **Potentially Interfering Substances Tested**

Interfering Substance	Test Concentration
Advil®	25 µg/mL
Amoxicillin	25 µg/mL
Benadryl®	350 ng/mL
Blood	5% v/v
Cepacol®	5 mg/mL
Chloraseptic® Sore throat (lozenges)	5 mg/mL
Chloraseptic® Sore throat (spray)	5% v/v
Chlor-Tripolon®	25 ng/mL
Dequadin®	12.5 µg/mL
Erythromycin	15 µg/mL
Listerine® (mouth wash)	5% v/v
Scope® (mouth wash)	5% v/v
Purified Mucin Protein[1]	4 mg/mL
NyQuil® COMPLETE[2]	0.5% v/v
Penicillin	1.2 mg/mL
Ricola®	5 mg/mL
Saline nasal spray	5% v/v
Saliva	5% v/v
Strepsils® extra	5 mg/mL
Sucrets® Complete	5 mg/mL
Toothpaste	0.1 mg/mL
Tylenol®	100 µg/mL
Zinc Lozenges	0.1 mg/mL

[1] Invalid results may be obtained in the presence of mucin at ≥5 mg/mL.

[2] False negative results may be obtained in the presence of NyQuil® (0.5% v/v).

Carry-Over and Cross Contamination

Carry-over and cross contamination for the ARIES® Group A Strep Assay was evaluated by testing thirty (30) high GAS positive samples in series, alternating with thirty (30) GAS negative samples consisting of Negative Simulated Matrix (NSM) only. The high positive samples were run adjacent to negative samples in an alternating pattern across ten (10) consecutive runs using one ARIES® System. No carry-over or cross contamination was observed, and the overall percent agreement with the expected results was 100% for both high positive and negative samples.

Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the assay was evaluated against nine (9) *Streptococcus pyogenes* strains that are different from those included in the LoD study. Each strain was diluted to an initial concentration of three times (3X) the confirmed LoD in negative simulated matrix (NSM) and tested in triplicate. The results showed that 8 out of 9 strains were detected with 100% positivity (in 3/3 replicates) in the initial testing at 3x LoD (*Table 8*); and the remaining 1 strain achieved 100% positivity (in 3/3 replicates) in testing at 5x LoD of the assay.

TABLE 8. **ARIES® Group A Strep Assay Analytical Reactivity (Inclusivity) Results**

***S. pyogenes* Strain**	**Source**	**Catalog #**	**100% Positivity Concentration (CFU/mL)**
Z018	ZeptoMetrix	0801512	1.24E+04
M-4, MGAS 10750 [FL01-86]	ATCC	BAA-1066	1.24E+04
M-6, MGAS 10394	ATCC	BAA-946	1.24E+04
QC A62	ATCC	49399	1.24E+04
M-38, Typing strain C94 [13RS1]	ATCC	12370	1.24E+04
M-89, CDC-SS-1397 [NCTC 12067, PT-4245, R81/1352]	ATCC	700949	1.24E+04
Typing strain T11	ATCC	12352	1.24E+04
M-1, Typing strain T1 [NCIB 11841, SF 130]	ATCC	12344	1.24E+04
M-3, Typing strain C203 [Dochez 1708]	ATCC	12384	2.07E+04

Analytical Specificity (Cross Reactivity and Microbial Interference)

Analytical specificity for the ARIES® Group A Strep Assay was assessed with 35 potentially cross-reacting organisms (CROs, *Table 9*) that may be present in throat swab specimens. Cross reactivity was evaluated by spiking potential CROs in negative simulated matrix (NSM) to a final concentration of 10^6 CFU/mL (or the highest available concentration) and testing in triplicate (n=3) on the ARIES® System. All 35 microorganisms tested for cross reactivity yielded GAS negative results and are therefore considered non-cross reactive with the ARIES® Group A Strep Assay (*Table 9*), in the absence of GAS. Microbial interference was evaluated by preparing CROs to a final concentration of 10^6 CFU/mL (or the highest available concentration) in NSM containing GAS culture prepared to 3x LoD. In initial testing, 34 potential CROs tested in the presence of GAS yielded the expected positivity for GAS while one organism, *Treponema denticola*, generated 2/3 GAS negative results. Repeat testing of *T. denticola* at approximately half the original concentration gave 100% (3/3) GAS positivity. False negative results may therefore be observed in the presence of high concentrations of *T. denticola*. There was no evidence of the potential for cross-reaction or interference with any of the other organisms tested.

TABLE 9. **ARIES® Group A Strep Assay Organisms Tested for Cross Reactivity and Microbial Interference**

#	Cross Reacting Organisms
1	*Arcanobacterium haemolyticum*
2	*Bacillus cereus*
3	*Bordetella pertussis*
4	*Burkholderia cepacia*
5	*Campylobacter rectus* *
6	*Candida albicans*
7	*Corynebacterium diphtheria*
8	*Enterococcus faecalis*
9	*Escherichia coli*
10	*Fusobacterium necrophorum*
11	*Haemophilus influenza*
12	*Klebsiella pneumoniae*
13	*Lactobacillus acidophilus*
14	*Moraxella catarrhalis*
15	*Neisseria gonorrhoeae*
16	*Parvimonas micra*
17	*Prevotella oralis*
18	*Pseudomonas aeruginosa*
19	*Saccharomyces cerevisiae*
20	*Staphylococcus aureus*
21	*Staphylococcus epidermidis*
22	*Streptococcus agalactiae*
23	*Streptococcus anginosus*
24	*Streptococcus canis*
25	*Streptococcus constellatus subsp. pharyngis*
26	*Streptococcus dysgalactiae subsp. equisimilis*
27	*Streptococcus gallolyticus*
28	*Streptococcus intermedius*
29	*Streptococcus mitis*
30	*Streptococcus mutans*
31	*Streptococcus pneumoniae*
32	*Streptococcus salivarius*
33	*Streptococcus sanguinus*

#	Cross Reacting Organisms
34	*Treponema denticola*†, ‡
35	*Veillonella parvula*

* The final testing concentration for *Campylobacter rectus* was ≥ 4.55 x 10^3 CFU/mL.

† No titer information available. Growth in transparent film, no colony formation.

‡ During initial testing of *Treponema denticola* in the presence of GAS at 3x LoD, 2 out of 3 replicates were GAS Negative. Repeat testing at approximately half the concentration of *T. denticola* gave 100% (3/3) GAS positive results.

Additional *in silico* analysis was performed to evaluate the potential for cross-reaction of the ARIES® Group A Strep Assay primers with microorganisms and viruses that may be found in throat swab specimens. No significant sequence similarity was observed that was predicted to produce false positive or false negative results.

Multi-Site Reproducibility

Reproducibility of the assay was evaluated by testing one lot of ARIES® Group A Strep Assay cassettes by two operators at each of the three sites on five non-consecutive days. A blinded reproducibility panel, consisting of a GAS low positive (1X LoD), a GAS moderate positive (3X LoD), and a negative sample, was prepared by an independent operator. The reproducibility panel was tested in triplicate by each operator on each day of testing. The results of the reproducibility study are shown in *Table 10*.

TABLE 10. **ARIES® Group A Strep Assay Site-to-Site Reproducibility Results**

Level	Positive/Number (%)			
	Site 1	Site 2	Site 3	Overall
Moderate Positive 3X LoD	30/30[1] (100)	29/30 (96.7)	30/30 (100)	89/90 (98.9)
Low Positive 1X LoD	29/30 (96.7)	30/30[1] (100)	28/30[2] (93.3)	87/90 (96.7)
Negative	0/30 (0.0)	1/30 (3.3)	0/30 (0.0)	1/90 (1.1)

[1] 1/30 samples was reported as Invalid on initial testing; reported as Positive upon repeat.

[2] All of 6 additional replicates that were tested were reported as Positive (overall 34/36 replicates, 94.4% were reported Positive at 1X LoD)

Within Laboratory Precision/Repeatability

The within-laboratory precision / Repeatability of the ARIES® Group A Strep Assay was evaluated by two operators performing testing on a single ARIES® System using a single lot of ARIES® Group A Strep Assay cassettes. The sample panel was prepared containing moderate positive (3X LoD), low positive (1X LoD), and negative samples that were blinded to operators with respect to the expected GAS concentration. A minimum of three replicates of each sample concentration were run at least five times by each operator. The results of the study are shown in *Table 11*.

TABLE 11. **ARIES® Group A Strep Assay Within-Laboratory Precision/Repeatability Results**

Level	Positive/Tested (%)
Moderate Positive 3X LoD	30/30 (100)
Low Positive 1X LoD	28/30 [1] (93.3)
Negative	0/30 (0.0)

[1] All of 12 additional replicates that were tested were reported as Positive (overall 40/42 replicates, 95.2% were reported Positive at 1X LoD)

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