DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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October 30, 2017

LUMINEX CORPORATION
JENNIFER GRIMES
INTERIM MANAGER, REGULATORY AFFAIRS
12212 TECHNOLOGY BLVD.
AUSTIN TX 78727

Re: K172402
Trade/Device Name: ARIES Group A Strep Assay, ARIES Group A Strep Assay Protocol File Kit
Regulation Number: 21 CFR 866.2680
Regulation Name: *Streptococcus* spp. nucleic acid-based assay
Regulatory Class: II
Product Code: PGX, OOI
Dated: August 8, 2017
Received: August 9, 2017

Dear Ms. Grimes:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K172402

Device Name
ARIES Group A Strep Assay

Indications for Use *(Describe)*
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 beta-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.

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

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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11.0 510(k) Summary

This Executive Summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

A. 510(k) Number:

K172402

B. Purpose for Submission:

Traditional 510(k), New Device

C. Measurand:

Streptococcus pyogenes (Group A β-hemolytic Streptococcus)

D. Type of Test:

Qualitative Real Time Polymerase Chain Reaction (PCR)

E. Applicant:

Jennifer Grimes
Luminex Corporation
12212 Technology Blvd
Austin, TX 78727

Tel: (416) 593-4323

F. Date Prepared

October, 2017

G. Proprietary and Established Names:

ARIES® Group A Strep Assay

H. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
PGX	Class II	21 CFR 866.2680 - *Streptococcus spp*. nucleic acid based assay	Microbiology (83)

I. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   For use with the ARIES® Systems.

J. Device Description:

The ARIES® Group A Strep Assay is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test system that consists of the ARIES® System or the ARIES® M1 System with their included ARIES® Software, an assay-specific cassette, and an assay-specific protocol file. The ARIES® Group A Strep Assay cassette is a disposable, single-use cassette containing nucleic acid purification reagents, internal sample process control (SPC), and an assay-specific master mix capable of performing the designated assay on one sample. The ARIES® Group A Strep Assay cassette directly detects *Streptococcus pyogenes* (Group A β-hemolytic Streptococcus) in throat swab specimens collected from the surface of human tonsils and posterior pharyngeal wall.

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 specimen is lysed and nucleic acid is extracted using an ARIES® System. An extractable sample processing control (SPC) target is present in the ARIES® Group A Strep Assay cassette and is processed with the specimen. The SPC controls for recovery of extracted nucleic acid, the presence of inhibitory substances and for PCR reagent and instrument integrity. The Ct value of the SPC is designed to verify nucleic acid extraction, to identify PCR inhibition, if any, and verify proper function of the extraction system and real-time instrument. The T_m value of the SPC is used as a reference for determining the target T_m.

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

K. Substantial Equivalence Information:

1. Predicate device name(s):

   Liat™ Strep A Assay (Manufactured by IQuum, Inc.)

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

   K141338

3. Comparison with predicate:

   The following tables compares the ARIES® Group A Strep Assay to IQuum, Inc.'s Liat™ Group A Strep Assay (K141338). Table 11-1 shows similarities between the new device and the predicate, while Table 11-2 shows the differences.

Table 11-1: Similarities between New Device and Predicate

Similarities		
Attribute	**New Device**	**Predicate Device (K141338)**
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.	The Liat™ Strep A Assay, performed on the Liat™ Analyzer, is a qualitative in vitro diagnostic test for the detection of *Streptococcus pyogenes* (Group A β-hemolytic Streptococcus, Strep A) in throat swab specimens from patients with signs and symptoms of pharyngitis. The Liat™ Strep A Assay utilizes nucleic acid purification and polymerase chain reaction (PCR) technology to detect *Streptococcus pyogenes* by targeting a segment of the *Streptococcus pyogenes* genome.
Sample type	Throat swabs (TS)	Throat swabs (TS)
Assay results	Qualitative	Qualitative
Assay format	Real-time PCR	Real-time PCR
Strep A. Target	Conserved region of Group A *Streptococcus* genome	Conserved region of Group A *Streptococcus* genome
Extraction Method	Automated with the ARIES® Systems	Automated with the Liat™ Analyzer

Table 11-2: Differences between New Device and Predicate

Differences		
Attribute	**New Device**	**Predicate Device (K141338)**
Assay instrument	ARIES System or ARIES M1 System	Liat™ Analyzer
Controls	Extractable Sample Processing Control (SPC)	Inactivated bacterial Internal Process Control (IPC)
Detection chemistry	Fluorescently labeled primers with quencher-labeled nucleotides; decrease in fluorescence over time	Fluorescently-labeled hydrolysis probes; increase in fluorescence over time
Result Interpretation	Ct values coupled with melt curve analysis	Ct and endpoint fluorescence values
Time-to-result	~2 hours	~15 minutes
Run size	1-12 samples with the ARIES System 1-6 samples with the ARIES M1 System	1 sample with the Liat™ Analyzer

L. Standards/Guidance Documents Referenced:

Not applicable.

M. Test Principle:

The ARIES Group A Strep Assay is based on an expanded genetic alphabet technology, consisting of synthetic DNA base pair 2'-deoxy-5-methyl-isocytidine (iC): 2'-deoxyisoguanosine (iG). The isobases (iC and iG) pair specifically with each other and

not with natural nucleotides. In addition, isobases are efficiently incorporated during PCR. During PCR amplification, a quencher-modified iGTP is incorporated by the polymerase opposite an iC and a fluorophore reporter attached to a PCR primer. If target is present and is amplified, assay fluorescence decreases with every cycle as amplification product accumulates. The decrease in assay fluorescence is monitored in real time using the ARIES System. Following PCR, the amplification products are thermally denatured and assay fluorescence is monitored. The strands of the amplification products are separated and assay fluorescence increases, thus enabling determination of the melting temperature (T_m) of the amplicon.

N. Performance Characteristics:

1. Analytical performance:

a. Reproducibility/Precision/Repeatability:

Reproducibility of the ARIES® Group A Strep (GAS) Assay was evaluated by testing one lot of ARIES GAS Assay cassettes by two operators at each of 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 11-3.

Table 11-3. ARIES® Group A Strep Assay Site-to-Site Reproducibility

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)

Lot-to-lot reproducibility of the ARIES® Group A Strep Assay was evaluated by one operator using a single ARIES system to test three lots of ARIES GAS Assay cassettes. A reproducibility panel was prepared containing moderate positive (3X LoD), low positive (1X LoD) and negative samples. A minimum of three replicates of each sample concentration were run a minimal five times on each cassette lot. The identity of these samples was blinded to the operator. The results of the study are shown in Table 11-4.

Table 11-4: ARIES® Group A Strep Assay Lot-to-Lot Reproducibility Results

Level	Positive/Tested (%)			
	Lot 1	Lot 2	Lot 3	Overall
Moderate Positive 3X LoD	15/15 (100)	15/15 (100)	15/15 (100)	45/45 (100)
Low Positive 1X LoD	14/15 (93.3)	15/15 (100)	13/15 (86.7)	42/45 (93.3) [1]
Negative	0/15 (0.0)	0/15 (0.0)	0/15 (0.0)	0/45 (0.0)

[1] All of 18 additional replicates that were tested (6 per reagent lot) were reported as Positive (overall 60/63 replicates, 95.2% reported Positive at 1X LoD)

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 GAS Assay cassettes. A reproducibility panel was prepared containing moderate positive (3X LoD), low positive (1X LoD) and negative samples, which were blinded to operators with respect to expected GAS concentration. A minimum of three replicates of each sample concentration were run a minimal five times by each operator. The results of the study are shown in Table 11-5.

Table 11-5: 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)

b. Linearity/assay reportable range:

Not applicable. The ARIES® Group A Strep Assay is a qualitative assay.

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

Stability:

Specimen Stability

Fresh specimen stability was assessed by testing specimens prepared at three times the Limit of Detection (3x LoD) in GAS-Negative Clinical Matrix (NCM) and stored at two different temperatures: ambient (20-25°C) and refrigerated (4-8°C). Following T0 testing (the same day of specimen preparation), specimens stored at ambient temperature were tested at 24 and 48 hours; and specimens stored at 4-8°C were tested at 24 and 48 hours, 4 days and 7 days.

Data showed that GAS positive and GAS negative specimens stored at both temperatures (ambient and 4-8°C) generated expected 100% GAS positive and 100% GAS negative results, respectively, for all time points tested (Table 11-6). These results indicate that GAS specimens are stable for up to 48 hours when stored at 20-25°C or for up to seven days at 4°C to 8°C.

Table 11-6: ARIES® Group A Strep Assay Fresh Specimen Stability Results

Target type	Time point	Agreement with Expected Results[a, b]	
		Ambient	4-8°C
GAS in NCM (3x LoD)	T0	100% (20/20)[c]	
	24 hours	100% (20/20)	100% (20/20)
	48 hours	100% (20/20)	100% (20/20)
	4 days		100% (20/20)
	7 days		100% (20/20)
NCM only	T0	100% (2/2)[c]	
	24 hours	100% (2/2)	100% (2/2)
	48 hours	100% (2/2)	100% (2/2)
	4 days		100% (2/2)
	7 days		100% (2/2)

[a] Expected result for GAS 3x LoD diluted in NCM is 100% GAS positive; expected result for the NCM only is 100% GAS negative.
[b] An overall invalid rate of 0.6% (1/176) was observed from fresh specimen testing results.
[c] T0 is defined as the same day that the contrived specimens were prepared, aliquoted and tested before storing at the appropriate temperatures.

Frozen specimen stability was assessed by testing ARIES® Group A Strep Assay culture prepared at three times the Limit of Detection (3x LoD) in GAS-Negative Clinical Matrix (NCM) and GAS-Negative Simulated Matrix (NSM) and stored at ≤ -70°C. Following T0 testing for specimens prepared in NCM or NSM, specimens stored at ≤ -70°C were tested at 1 month, 3 months and 6 months, with remaining time points to be tested at 9 and 12 months.

Data collected up to 6 months showed that GAS positive and GAS negative specimens stored at ≤ -70°C generated 100% GAS positive and 100% GAS negative results, respectively, for all time points tested. Results from frozen specimen

testing with the ARIES GAS assay are summarized in Table 11-7. Test results obtained to-date indicate that frozen specimens are stable for up to 6 months when stored at ≤ -70°C.

Table 11-7: ARIES® Group A Strep Assay Frozen Specimen Stability Results

Target type	Time point	Agreement with Expected Results[a, b]
GAS in NCM (3x LoD)	T0	100% (20/20)[c]
	1 month	100% (20/20)
	3 month	100% (20/20)
	6 month	100% (20/20)
GAS in NSM (3x LoD)	T0	100% (20/20)[c]
	1 month	100% (20/20)
	3 month	100% (20/20)
	6 month	100% (20/20)
NCM only	T0	100% (2/2)[c]
	1 month	100% (2/2)
	3 month	100% (2/2)
	6 month	100% (2/2)
NSM only	T0	100% (2/2)[c]
	1 month	100% (2/2)
	3 month	100% (2/2)
	6 month	100% (2/2)

[a] Expected result for GAS 3x LoD diluted in NCM or NSM is 100% GAS positive; expected result for the NCM or NSM only is 100% GAS negative.

[b] An overall invalid rate of 0.6% (1/177) was observed for this study for data collected up to the 6 month time point.

[c] T0 is defined as the same day the contrived specimens were prepared, aliquoted and tested before storing at the appropriate temperature.

Shelf-Life Stability

A real time stability study was performed to evaluate the shelf life of ARIES GAS cassettes. Stability was assessed by testing 6 replicates of ARIES Extractable GAS Control (100X LoD) and negative targets (Copan Liquid Amies Medium) on three different lots of ARIES GAS cassettes stored at 2 different temperatures, 4°C (2 – 8°C) and room temperature (15 – 30°C), at 10 different time points extending out to 19 months. The study was designed following guidelines listed in EP25-A, *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved.* Acceptance criteria for stability at each time point and temperature was established as 100% positivity for all GAS replicates and 100% negativity for all negative replicates. Data collected up to 7 months gave expected results indicating stability of the ARIES GAS cassettes up to 6 months

under the recommended storage conditions (with a 1 month safety margin). This study is on-going and will be updated as more data are collected.

Controls:

Process Control

Each ARIES® Group A Strep Assay cassette contains a Sample Process Control (SPC), which is processed with the sample and analyzed during the amplification reaction. The SPC verifies nucleic acid extraction, and proper reagent, cassette, ARIES® System, and assay protocol performance. The SPC has a known melting temperature (T_m) range and Ct range. Each time an assay is run, the system measures the temperature and fluorescence intensity of the SPC control to ensure the thermal and optical subsystems have remained in calibration.

External Controls

External controls should be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. A reference *Streptococcus pyogenes (S. pyogenes)* strain or well characterized *S. pyogenes* clinical isolate 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 *S. pyogenes* may be used as Positive and Negative External Controls, respectively. The ARIES® Group A Strep Assay Cassette Kit does not include external positive and negative controls.

d. Detection Limit:

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES® Group A Strep Assay using two strains of *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 11-8.

Table 11-8: 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%

e. Analytical Reactivity (Inclusivity)

The analytical reactivity (inclusivity) of the ARIES® Group A Strep Assay was evaluated against nine (9) *Streptococcus pyogenes* strains that are different from those included in the Limit of Detection (LoD) study. Each strain was diluted to an initial concentration of three times (3X) the confirmed LoD in GAS-negative simulated matrix (NSM) and tested in triplicate. The results showed that 8 out of 9 strains were detected with 100% positivity (3/3 replicates) in the initial testing at 3x LoD; and the remaining 1 strain achieved 100% positivity (3/3 replicates) in testing at 5x LoD of the assay. The study results are shown in Table 11-9.

Table 11-9: 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

f. Analytical specificity:

Interfering Substances:

The potential inhibitory 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 limit of detection (LoD) in simulated throat swab matrix that was spiked with the highest potential concentration of each substance. The expected results were obtained in all cases except in the presence of NyQuil and mucin. With NyQuil at 0.5% (v/v), 2/3 samples produced false negative results on initial testing, although repeat analysis under the same conditions produced the expected results. Invalid results were observed in the presence of mucin at >4mg/mL.

Table 11-10. 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 >4g/mL

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

Cross Reactivity/Microbial Interference:

Analytical specificity for the ARIES® Group A Strep Assay was assessed with 35 microorganisms (Table 11-11) which may be present in throat swab specimens.

Cross reactivity was evaluated by spiking each potentially cross-reactive organism (CRO) in GAS-negative simulated matrix (NSM) to a final concentration of 10^6 CFU/mL (or the highest available concentration) and by 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-reactive with the ARIES GAS Assay. Microbial interference was evaluated by preparing each CRO to a final concentration of 10^6 CFU/mL (or the highest available concentration) in NSM containing GAS culture prepared to 3 times the Limit of Detection (3x LoD). In the initial testing, 34 CROs tested in the presence of GAS yielded the expected positivity for GAS while one CRO, *Treponema denticola*, generated 2/3 GAS negative results. Repeat testing of this CRO at ~50% lower concentration gave 100% (3/3) GAS positivity (Table 11-11). Therefore, among the potential CROs tested with the ARIES GAS Assay, interference was only observed with high concentrations of *T. denticola*.

In addition to the 35 microorganisms tested on the ARIES System, 16 microorganisms and viruses were tested for potential cross reactivity by *in silico* analysis (Table 11-12). Two *Pseudomonas* spp., *P. fluorescens* LBUM223 and *P. fluorescens* KENGFT3, were at the borderline of the cut-off criteria for potential cross reactivity. Culture materials for these two organisms were not commercially available to evaluate with the ARIES GAS Assay; however, *Pseudomonas aeruginosa,* a species of *Pseudomonas* associated with respiratory infections in humans, showed no cross reactivity when tested with the ARIES GAS assay. Overall, the results of the *in silico* analysis showed that the potential for cross-reaction or interference with the ARIES GAS Assay is low.

Table 11-11. ARIES® Group A Strep Assay Cross Reactivity and Microbial Interference Microorganisms Tested

#	Cross Reacting Organisms
1	*Arcanobacterium haemolyticum*
2	*Bacillus cereus*
3	*Bordetella pertussis*
4	*Burkholderia cepacia*
5	*Campylobacter rectus**
6	*Candida albicans*

#	Cross Reacting Organisms
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*
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 ~50% of the initial concentration gave 100% (3/3) GAS positivity.

Table 11-12. Microorganisms tested for Cross Reactivity/Microbial Interference *in silico*

Genus and Species/Names			
1	*Candida spp.*	9	Adenovirus Type 1
2	*Enterococcus spp.*	10	Adenovirus Type 7
3	*Klebsiella spp.*	11	Human influenza virus A
4	*Lactococcus lactis*	12	Human influenza virus B
5	*Legionella spp.*	13	Human parainfluenza virus
6	*Mycoplasma pneumoniae*	14	Human metapneumovirus
7	*Pseudomonas spp.*	15	Respiratory syncytial virus Type B
8	*Stenotrophomonas maltophilia*	16	Rhinovirus

Carry-Over/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 GAS-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. The results of this study show that no carry-over or cross contamination was observed, and the overall percent agreement with expected results was 100% for both high positive and negative samples.

g. Assay cut-off:

For the ARIES® Group A Strep Assay, the target (*sdaB*) has a Ct cut-off, Tm window, and Tm Peak Threshold. In addition, the internal sample process control (SPC) also has a corresponding Ct cut-off, Tm window, and Tm Peak Threshold. Collectively, the cut-off values compose the assay protocol file parameters, which are used to determine the assay result for the detection target as POSTIVE, NEGATIVE, or INVALID. These values are hard-coded into the ARIES® Group A Strep Assay protocol file and are not modifiable. The Assay Protocol File parameters were determined, and their performance in the ARIES® Group A Strep Assay was evaluated according to the following general procedure:

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

The assay parameters for the ARIES® Group A Strep Assay are considered **confidential and proprietary**.

2. Comparison Studies:

a. Method comparison with predicate device:

Not applicable.

b. Collection Media Comparison:

Not applicable.

c. Swab Comparison:

Not applicable.

3. Clinical Performance:

The clinical performance of ARIES® Group A Strep Assay was evaluated using de-identified, throat swab specimens prospectively collected from patients suspected of having respiratory infection attributable to *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*). Specimen collection was performed using the Copan or BD Liquid Amies Elution Swab (ESwab) Collection and Transport System.

Four (4) geographically distinct clinical sites within the United States prospectively collected specimens from January 2017 to May 2017 using the ARIES® System. All eligible clinical specimens were tested by both the reference method (bacterial culture followed by organism identification by Matrix-Assisted Laser Desorption/Ionization - Time-of-Flight Mass Spectrometry (MALDI-TOF MS)) and the ARIES® Group A Strep Assay and the results compared. Reference method testing was performed at a centralized testing facility while 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 analysis of performance 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.

The ARIES® Group A Strep Assay Performance compared to bacterial culture followed by identification with MALDI-TOF MS is summarized in Table 11-13 below. 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 11-13: 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 followed by identification with MALDI-TOF MS. All five (5) specimens were excluded from the device performance calculations.

The study results demonstrate that the diagnostic accuracy of the ARIES® Group A Strep Assay is acceptable for the detection of *Streptococcus pyogenes* in throat swab specimens from patients with signs and symptoms of pharyngitis.

4. Expected values:

During clinical evaluation of the assay, 26.8% (167/623) of throat swab specimens were reported as positive for Group A Strep by the ARIES® Group A Strep Assay. The prevalence of Group A Strep with the ARIES® Group A Strep Assay summarized by age groups and by gender in Table 11-14 and Table 11-15 below.

Table 11-14: ARIES® Group A Strep Assay Expected Values by Age Group

Age (yrs)	Total Positive by ARIES®	Expected Value
<2	3	25.0% (3/12)
2 - 11	135	32.5% (135/416)
12 - 21	21	12.9% (21/163)
22 - 59	8	28.6% (8/28)
≥60	0	0.0% (0/4)
Overall	**167**	**26.8% (167/623)** [1]

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

Table 11-15: ARIES® Group A Strep Assay Expected Values by Gender

Gender	Total Positive by ARIES	Expected Value
Male	82	28.8% (82/285)
Female	85	25.1% (85/338)
Overall	**167**	**26.8% (167/623)** [1]

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

O. Proposed Labeling:

The labeling provided in the submission satisfies the requirements of 21 CFR 809.10.

P. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.