510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K172402

B. Purpose for Submission:

To obtain a Substantial Equivalence Determination for the ARIES Group A Strep Assay

C. Measurand:

Conserved region of the *Streptococcus pyogenes sdaB* gene

D. Type of Test:

Qualitative real-time Polymerase Chain Reaction (PCR)

E. Applicant:

Luminex Corporation

F. Proprietary and Established Names:

ARIES Group A Strep Assay
ARIES Group A Strep Assay Protocol File Kit

G. Regulatory Information:

1. Regulation section:

21 CFR 866.2680: *Streptococcus* spp. nucleic acid-based assay

2. Classification:

Class II

3. Product code:

PGX: Groups A, C and G β-Hemolytic *Streptococcus* Nucleic Acid Amplification System

OOI: Real time nucleic acid amplification system

4. Panel:

   83-Microbiology

H. 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.

   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).

4. Special instrument requirements:

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

I. Device Description:

The ARIES Group A 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 the associated ARIES Software and an assay-specific Protocol File. The ARIES Group A Assay cassette is a disposable, single-use device that contains nucleic acid purification reagents, an internal Sample Processing Control (SPC), and an assay-specific master mix for the detection of a conserved region of the *S. pyogenes sdaB* gene. The assay is for use on throat swab specimens from patients with signs and symptoms of pharyngitis.

Throat swab specimens are collected and transported to the testing laboratory using the

Copan or BD Liquid Amies Elution Swab (ESwab) Collection and Transport System (nylon flocked swab with 1mL of liquid Amies medium). An aliquot of the transport medium is added directly to the assay cassette which is loaded into the ARIES instrument for automated nucleic acid extraction, amplification and detection.

The assay cassette includes a Sample Processing Control (SPC) that is extracted and processed with the patient specimen. The SPC is designed to monitor DNA recovery, amplification and detection.

Extracted nucleic acid is transferred through the assay cassette by magnetic beads and the eluted sample is then used to rehydrate lyophilized PCR reagents that are specific for the *S. pyogenes sdaB* gene and the SPC. Each primer pair is labeled with a different fluorophore and is detected in a different optical channel of the ARIES Systems. During PCR amplification, synthetic quencher nucleotides are incorporated into the amplified products that result in a decrease in fluorescence in the corresponding optical channel when target DNA is present. Following amplification, melt curve analysis is performed to confirm the identity of the amplicons generated. Results are interpreted automatically using parameters in the ARIES Group A Strep Assay Protocol File as either "Group A *Streptococcus* Positive", "Group A *Streptococcus* Negative" or "Invalid", and may be reported from the ARIES Software or from the optional SYNCT Software desktop application.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Liat Strep A Assay

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

   K141338

3. Comparison with predicate:

Similarities		
Item	**Device (K172402)**	**Predicate (K141338)**
	ARIES Group A Strep Assay	**Liat Strep A Assay**
Regulation	21 CFR 866.2680	Same
Product Code	PGX	Same
Device Class	Class II	Same
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.
Analyte	Group A *Streptococcus*	Same
Measurand	Conserved region of the *S. pyogenes* genome	Same
Specimen Type	Throat swab in liquid Amies medium	Same
Assay Format	Unitized ready for use	Same
External Controls	Available	Same
Result	Qualitative	Same

Differences		
Item	**Device (K172402)**	**Predicate (K141338)**
	ARIES Group A Strep Assay	**Liat Strep A Assay**
Instrument System	ARIES System or ARIES M1 System	Liat Analyzer
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	Cycle threshold value coupled with melt curve analysis	Cycle threshold and endpoint fluorescence values in addition to other parameters
Time to Result	~2 hours	~15 minutes

K. Standard/Guidance Document Referenced (if applicable):

CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline -Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI. *Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.* CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.

CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition.* CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

CLSI. *User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edition*. CLSI document EP15-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition.* CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

CLSI. *Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline - Second Edition.* CLSI document EP24-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

CLSI. *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.* CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

CLSI. *Molecular Diagnostic Methods for Infectious Diseases – Third Edition*. CLSI report MM03. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

CLSI. *Abbreviated Identification of Bacteria and Yeast: Approved Guideline - Second Edition*. CLSI document M35-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

ISO 14971. Medical devices - Application of risk management to medical devices.

L. Test Principle:

The ARIES Group A Strep Assay is performed on throat swabs from patients with signs and symptoms of pharyngitis. Specimens are collected using the Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System. Upon receipt in the testing laboratory, the ESwab tube is vortexed to mix the contents, and 200µL of the transport medium is then added to an ARIES Group A Strep Assay cassette which is loaded into the magazine of the ARIES System for automated processing. Up to 6 cassettes can be loaded in a single magazine. Within the instrument, a barcode on the ARIES Group A Strep Assay cassette is automatically scanned to associate the cassette with the appropriate assay protocol that provides all the necessary information to perform the test, analyze the data and generate the result report.

Each assay cassette contains all the reagents needed for nucleic acid extraction, amplification and detection for one sample and includes a Sample Processing Control (SPC) to monitor reagent and process integrity. Processing of the sample within the assay cassette is fully automated and there is no direct contact between the sample or reagents and the instrument, thereby reducing the potential for contamination.

The extracted nucleic acids are used to rehydrate the lyophilized PCR Master Mix which contains specific, fluorescently labeled primers for the *S. pyogenes* target and the SPC. The primers contain the synthetic nucleotide base 2'-deoxy-5-methyl-isocytidine (iC). During PCR amplification, incorporation of quencher-modified 2'-deoxyisoguanosine triphosphate (iG) into the nascent DNA strand opposite the iC residues results in a target-specific reduction of fluorescence with each successive cycle as amplicons accumulate. The ARIES Systems monitor the decrease in fluorescence in real-time and use the changes in signal over the course of the reaction to calculate amplification cycle (Ct) values. At the end of the reaction the amplification products undergo melt curve analysis to verify their identity. A combination of metrics for the *S. pyogenes* gene target and the SPC is used for result interpretation. If the *S. pyogenes* target is detected, the sample is reported as "Group A *Streptococcus* Positive". If the *S. pyogenes* target is not detected but the SPC signal is valid, the sample is reported as "Group A *Streptococcus* Negative", otherwise the result is "Invalid".

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Site-to-Site Reproducibility
The reproducibility of the ARIES Group A Strep Assay between sites was evaluated in a study performed by two operators at each of three sites over a period of five days. Each operator tested a blinded panel of *S. pyogenes* positive and negative samples using the same lot of reagents (3 sites X 2 operators X 5 days X 3 replicates = 90 data points per panel member). The panels were prepared using simulated throat swab matrix (**Section M(2)(b)**)

and *S. pyogenes* strain ATCC 700294. The results of the study demonstrated acceptable reproducibility from site-to-site at target levels close to the limit of detection (LoD) of the assay (**Table 1**).

Table 1. Summary of results from the ARIES Group A Strep Assay Site-to-Site Reproducibility Study, stratified by site and overall

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

[1] LoD: Limit of Detection for *S. pyogenes* strain ATCC 700294 (**Section M(1)(d)**)
3X LoD = 1.24 x 10^4 CFU/mL; 1X LoD = 4.13 x 10^3 CFU/mL

[2] 1/30 samples was reported as Invalid on initial testing; reported as Positive upon repeat

[3] All of 6 additional replicates that were tested at this target level were reported as Positive (overall at Site 3, 34/36 replicates (94.4%) were reported as Positive at 1X LoD)

Within Laboratory Precision/Repeatability

Within laboratory precision/repeatability of the ARIES Group A Strep Assay was evaluated by two operators who tested a panel of samples in triplicate on a single ARIES instrument over a period of 5 days (2 operators X 3 replicates X 5 days = 30 replicates per panel member). The panel members were the same as those used in the Site-to-Site Reproducibility Study, above. The results of the study demonstrated acceptable repeatability and precision from day-to-day with target levels close to the LoD of the assay (**Table 2**).

Table 2. Summary of results from the Within Laboratory Precision/Repeatability Study for the ARIES Group A Strep Assay

Level	Positive/Tested (%)
Moderate Positive 3X LoD [1]	30/30 (100)
Low Positive 1X LoD	28/30 [2] (93.3)
Negative	0/30 (0.0)

[1] LoD: Limit of Detection for *S. pyogenes* strain ATCC 700294 (**Section M(1)(d)**)
3X LoD: 1.24 x 10^4 CFU/mL; 1X LoD: 4.13 x 10^3 CFU/mL

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

Lot-to-Lot Reproducibility

The lot-to-lot reproducibility of the ARIES Group A Strep Assay was evaluated by testing a panel of *S. pyogenes* positive and negative samples in simulated throat swab matrix with each of three lots of reagents over a period of five days (3 replicates X 3 lots X 5 days = 45 replicates per panel member). The panel members were the same as those used in the

Reproducibility and Precision/Repeatability Studies, above. The results are summarized in Table 3 and show acceptable performance with each lot of reagents.

Table 3. Summary of results from the ARIES Group A Strep Assay Lot-to-Lot Reproducibility Study, stratified by reagent lot and overall

Level	Positive/Tested (%)			
	Lot 1	Lot 2	Lot 3	Overall
Moderate Positive 3X LoD [1]	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) [2]
Negative	0/15 (0.0)	0/15 (0.0)	0/15 (0.0)	0/45 (0.0)

[1] LoD: Limit of Detection for *S. pyogenes* strain ATCC 700294 (Section M(1)(d))
X LoD: 1.24 x 10^4 CFU/mL; 1X LoD: 4.13 x 10^3 CFU/mL

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

b. *Linearity/assay reportable range:*

Not applicable.

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

Specimen Stability
The stability of throat swabs for use with the ARIES Group A Strep Assay was evaluated analytically by testing pooled *S. pyogenes* negative Eswab swab specimens that were seeded with an enumerated suspension of cultured organisms and stored under different conditions. Unseeded Eswab throat matrix was included to assess the effect of specimen storage on the performance of the SPC. Twenty (20) *S. pyogenes* positive and two (2) *S. pyogenes* negative assay replicates were tested at each stability time point. The results of these studies support the stability of throat swabs for use with the ARIES Group A Strep Assay when collected using the ESwab Specimen Collection and Transport System for up to 7 days at 4-8°C, up to 48 hours at 20-25°C or up to 6 months when frozen at ≤-70°C.

Reagent Stability
The shelf-life of the ARIES Group A Strep Assay cassettes was evaluated in a real-time stability study performed on three lots of reagents that were stored either refrigerated (2-8°C) or at room temperature (15-30°C). The results from the study support assignment of an expiration date 7 months from the day of manufacture for the assay cassettes when stored under the recommended conditions.

Sample Processing Control
Each ARIES Group A Strep Assay cassette contains a Sample Processing Control (SPC) that is designed to monitor DNA recovery, amplification and detection. Samples that are negative for *S. pyogenes* by the ARIES assay and in which the SPC is not detected are reported as

"Invalid" and must be retested using the residual swab transport medium and a new test cassette.

External Controls
External Controls should be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. A reference strain of *S. pyogenes* or well characterized clinical isolate may be used as a Positive Control. ESwab transport medium may be used as a Negative Control. Alternatively, clinical specimens that are known to be positive or negative for *S. pyogenes* may be used as Positive and Negative External Controls, respectively.

External Positive and Negative Controls were tested on a daily basis during the prospective Clinical Study described in **Section M(3)(a)** using a total of four ARIES systems and three reagent lots. The Positive External Control comprised a standardized suspension of a strain of *S. pyogenes* ATCC 700294 at 1.24 x 10^4 CFU/mL (3X LoD). The Negative External Control comprised liquid Amies medium alone. On initial testing, 153/158 (96.9%) Positive and 163/163 (100%) Negative External Controls produced the expected results.

d. Detection limit:

Limit of Detection
The Limit of Detection (LoD) of the ARIES Group A Strep Assay was estimated for two strains of *S. pyogenes* by testing various dilutions of enumerated cell stocks in throat swab matrix. The LoD for each strain was then confirmed by testing a further 20 replicates at the lowest target level that produced 100% positive results. The LoD was defined as the lowest concentration tested at which ≥95% of assay replicates produced positive results. For ATCC 19615, the LoD was determined to be 2.58 x 10^3 CFU/mL and for ATCC 700294 it was 4.13 x 10^3 CFU/mL.

Inclusivity (Analytical Reactivity)
The inclusivity of the ARIES Group A Strep Assay was evaluated by testing nine strains of *S. pyogenes* in simulated throat swab matrix in addition to those included in the LoD Study (**Table 4**). Eight of the nine strains produced 3/3 positive results at a concentration of 1.24 x 10^4 CFU/mL (3X the LoD for ATCC 700294). Strain ATCC 12384 gave one false negative result at this target level but produced 3/3 positive results at 2.07 x 10^4 CFU/mL, equivalent to 5X LoD for ATCC 700294. These results are acceptable.

Table 4. Strains of *C. difficile* used to evaluate the inclusivity of the ARIES *C. difficile* Assay

Source	Strain Number	Antigenic Type
Zeptometrix	Z018	Not known
ATCC [1]	BAA-1066	M4
	BAA-946	M6
	12344	M1, T1
	12352	T11
	12370	M38
	12384	M3
	49399	Not known
	700949	M89

ATCC: American Type Culture Collection
[1] Antigenic types listed are as as reported by ATCC

Bioinformatic Analysis
The inclusivity of the ARIES Group A Strep primers for the targeted region of the genome was analyzed *in silico* using the Basic Local Alignment Search Tool (BLAST). The region was shown to be well conserved, with no evidence of sequence heterogeneity that could lead to false negative results. This is acceptable.

e. *Analytical specificity:*

Cross-reactivity Study
The analytical specificity of the ARIES Group A Strep Assay was evaluated by testing a panel of 35 organisms that may be found in throat swab specimens (**Table 5**). Each strain was tested in triplicate in simulated throat swab matrix at $\geq 10^6$ CFU/mL (or the highest concentration attainable). No false positive or Invalid results were obtained. These results are acceptable.

Table 5. Organisms tested for potential cross-reaction in the ARIES Group A Strep Assay

Bacteria	
Arcanobacterium haemolyticum	*Staphylococcus aureus*
Bacillus cereus	*Staphylococcus epidermidis*
Bordetella pertussis	*Streptococcus agalactiae*
Burkholderia cepacia	*Streptococcus anginosus*
Campylobacter rectus [1]	*Streptococcus canis*
Corynebacterium diphtheriae	*Streptococcus constellatus* subsp. *pharyngis*
Enterococcus faecalis	*Streptococcus dysgalactiae* subsp. *equisimilis*
Escherichia coli	*Streptococcus gallolyticus*
Fusobacterium necrophorum	*Streptococcus intermedius*
Haemophilus influenzae	*Streptococcus mitis*
Klebsiella pneumoniae	*Streptococcus mutans*
Lactobacillus acidophilus	*Streptococcus pneumoniae*
Moraxella catarrhalis	*Streptococcus salivarius*
Neisseria gonorrhoeae	*Streptococcus sanguinus*
Parvimonas micra [2]	*Treponema denticola* [4]
Prevotella oralis [3]	*Veillonella parvula*
Pseudomonas aeruginosa	
Yeast	
Candida albicans	*Saccharomyces cerevisiae*

[1] Tested at 4.55 x 10^3 CFU/mL (the highest available concentration)
[2] Formerly *Peptostreptococcus micros*
[3] Formerly *Bacteroides oralis*
[4] No titer available; tested at the highest available concentration

Bioinformatic Analysis

In silico analysis was performed to evaluate the potential for cross-reaction of the ARIES Group A Strep Assay primers with additional microorganisms and viruses that may be found in throat swab specimens (**Table 6**). No significant homology was observed that was predicted to produce false positive results.

Table 6. Organisms and viruses evaluated *in silico* for potential cross-reaction in the ARIES Group A Strep Assay

Viruses	**Bacteria**
Human Adenovirus 1	*Enterococcus* spp.
Human Adenovirus 7	*Klebsiella* spp.
Influenza Virus A	*Lactococcus lactis*
Influenza Virus B	*Legionella* spp.
Human metapneumovirus 1	*Mycoplasma pneumoniae*
Human Parainfluenza virus 2	*Pseudomonas* spp.
Human Parainfluenza virus 3	*Stenotrophomonas maltophilia*
Human Parainfluenza virus 4	**Yeast**
Human Parainfluenza virus 4a	*Candida* spp.
Human Respiratory Syncytial Virus B	
Rhinovirus	

Contamination Study
The potential for false-positive results with the ARIES Group A Strep Assay due to within run or between run cross-contamination was evaluated by testing an alternating series of *S. pyogenes* "high positive" and negative samples in successive instrument runs. The high positive samples contained *S. pyogenes* at a concentration of 10^6 CFU/mL of simulated throat swab matrix. Negative samples comprised simulated throat swab matrix alone. The expected results were obtained for all *S. pyogenes* positive and negative samples (30/30 each). These results are acceptable.

f. Assay cut-off:

The ARIES Group A Strep Assay result algorithm uses a combination of parameters based on cycle threshold, amplicon melting temperature and fluorescence intensity for the *S. pyogenes* target and SPC to report results as either Group A *Streptococcus* Positive, Negative or Invalid. The algorithm parameters were established through Receiver Operator Characteristic (ROC) analysis using residual *S. pyogenes* positive and negative clinical specimens that were characterized using the same reference method as in the prospective clinical validation study described in **Section M(3)(a)**.

g. Assay interference:

Potentially Interfering Substances
The potential for interference with the ARIES Group A Strep Assay was evaluated with endogenous and exogenous substances that may be present in throat swab specimens (**Table 7**). Each substance was tested in triplicate in the presence and absence of *S. pyogenes* using samples prepared with simulated throat swab matrix (**Section M(2)(b)**). Invalid results were obtained in the presence of mucin at 5mg/mL although additional testing demonstrated no interference at a concentration of ≤4mg/mL. Two of three samples containing 0.5% NyQuil produced false negative results on initial testing, although repeat analysis under the same condition produced the expected results. The potential for interference with the ARIES Group A Strep Assay in the presence of NyQuil (0.5% v/v) and mucin (>4mg/mL) is noted in the device labeling.

Table 7. Substances evaluated for potential interference with the ARIES Group A Strep Assay

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

[1] In the presence of NyQuil (0.5% v/v), 2/3 *S. pyogenes* positive samples produced false negative results on initial testing; upon repeat analysis 3/3 replicates produced the expected results.

[2] When mucin was tested at 5mg/mL, 2/6 *S. pyogenes* positive samples and 3/6 *S. pyogenes* negative samples produced Invalid results (including initial and repeat testing). All results (15/15) obtained at 0.2-4mg/mL were as expected.

Microbial Interference

The potential for interference with the ARIES Group A Strep Assay by organisms that may be present in throat swab specimens was investigated using the same list of species that was evaluated for potential cross-reactivity (refer to **Section M(1)(e)** and **Table 5**). Testing was performed in triplicate with each potentially interfering species in the presence of *S. pyogenes* ATCC 700294 at 3X LoD. The potentially interfering species were tested at 10^6 CFU/mL of simulated throat swab matrix or the highest concentration available. The expected results were obtained with all the organisms tested with the exception of samples containing *Treponema denticola* for which 2/3 replicates produced false negative results. No titer was available for the *T. denticola* cell stock used in this study but retesting at a lower concentration (~50% of the original test concentration) produced the expected results. Although bioinformatic analysis showed no evidence of cross-reaction between the ARIES Group A Strep Assay primers and *T. denticola*, the potential for interference by this organism is noted in the Limitations section of the device labeling.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

Comparison of Performance with Natural and Simulated Matrices
To provide a sufficient quantity of material for testing, a simulated throat matrix was used for the majority of Analytical Studies. Testing with simulated matrix was performed in accordance with the standard assay procedure by transferring 200µL of the sample to the ARIES Group A Strep Assay cartridge.

The suitability of the simulated matrix for use in analytical testing was evaluated in a comparison study with natural clinical matrix. The two matrices were tested in parallel as part of the LoD Study described **Section M(1)(d)** using *S. pyogenes* strains ATCC 19615 and 700294. The results demonstrated similar analytical sensitivity in both matrices and there were negligible differences in relevant assay metrics for both the *S. pyogenes* target and SPC. The study therefore provided acceptable evidence to support the use of simulated matrix in the Analytical Studies to characterize the performance of the ARIES Group Strep A Assay.

3. Clinical studies:

a. Clinical Sensitivity:

The performance of the ARIES Group A Strep Assay was evaluated in a prospective multicenter study that was conducted at four (4) clinical sites in the U.S. Throat swab specimens were collected from subjects who presented with a signs and symptoms of pharyngitis for whom testing for Group A *Streptococcus* was ordered by their physician.

At three sites, swab specimens for use in the study were collected under informed consent, while at one site the study was performed using residual, de-identified specimens leftover from standard of care testing. All the specimens were collected using the Copan or BD Liquid Amies Elution Swab (ESwab) Collection and Transport System and refrigerated within 4 hours. The transport medium from each specimen was divided into four aliquots for use in different test methods: #1 (≥125µL), for shipment to a centralized laboratory for reference culture; #2 and #3 (≥250µL each), for testing with the ARIES Group A Strep Assay at the clinical sites (including repeat analysis, if required); #4 (≥200µL), stored frozen at ≤-70°C for analysis by PCR/bidirectional sequencing (only performed for subjects with discordant reference culture and ARIES Group A Strep results).

The reference culture procedure was performed within 48 hours of specimen collection and comprised inoculation of Group A Selective *Streptococcus* Agar and Trypticase Soy Agar (both containing 5% sheep blood) with 50µL ESwab transport medium and

incubation under anaerobic conditions for up to 48 hours. Culture plates that did not exhibit β-hemolytic colonies after 48 hours were recorded as negative for Group A *Streptococcus*. β-hemolytic colonies present on the primary plates were sub-cultured and identified by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonics, Inc.) according to the manufacturer's instructions.

Seven hundred and thirty-five (735) specimens were initially enrolled in the study. 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), failure to meet the inclusion criteria or eligibility for inclusion not confirmed (30), insufficient specimen volume (7), use of an incorrect collection swab or eligibility of the swab transport medium not confirmed (3), lack of a pure isolate (2), ARIES testing performed by an ineligible operator (2), or prior enrolment of the subject (1). In addition, five specimens gave inconclusive reference culture results (MALDI-TOF MS log(score) <2.00) and were excluded from the performance calculations. A total of 618 specimens were therefore included in the analysis of performance (**Table 8**). On initial testing, 6/623 specimens (1.0%) produced Invalid results. All 6 specimens were retested and produced valid results for a final Invalid rate of 0% (0/623).

Table 8. Expert ARIES Group A Strep Assay Clinical Performance vs Reference Culture

		Reference Culture		
		Positive	**Negative**	**Total**
ARIES Group A Strep Assay	**Positive**	156	10 [1]	**166**
	Negative	4 [2]	448	**452**
	Total	**160**	**458**	**618** [3]
Sensitivity		156/160 = 97.5% (95% CI: 93.7-99.0%)		
Specificity		448/458 = 97.8% (95% CI: 96.0-98.8%)		
Positive Predictive Value		156/166 = 94.0% (95% CI: 89.3-96.7%)		
Negative Predictive Value		448/452 = 99.1% (95% CI: 97.7-99.7%)		

95% CI: Two-sided 95% score confidence interval

[1] 7/10 specimens were positive by an alternative PCR/bi-directional sequencing assay

[2] 2/4 specimens were positive by an alternative PCR/bi-directional sequencing assay

[3] 5 specimens gave inconclusive reference culture results (MALDI-TOF MS log(score) <2.00 by the Direct, Extended Direct and Extraction test methods) and were excluded from the performance calculations

The performance of the ARIES Group A Strep Assay at each clinical site in comparison to the reference culture method is shown in **Table 9**. Although the number of residual specimens tested was small, a higher point estimate for sensitivity was observed in testing performed with specimens collected prospectively under informed consent (98.5%; 95% confidence interval 94.6-99.6%) than with residual specimens (92.9%, 95% CI: 77.4-98.0%). Overall, performance was determined to be acceptable.

Table 9. Performance of the ARIES Group A Strep Assay in comparison to the reference culture method, stratified by clinical site

Specimen Collection	Site Number	Culture Positive (%)	ARIES Group A Strep (%; 95% Score Confidence Interval)	
			Sensitivity	Specificity
Informed Consent [1]	**1**	37/102 (36.3)	36/37 (97.3; 86.2-99.5)	62/65 (95.4; 87.3-98.4)
	2	60/238 (25.2)	59/60 (98.3; 91.1-99.7)	175/178 (98.3; 95.2-99.4)
	3	35/130 (26.9)	35/35 (100; 90.1-100)	94/95 (98.9; 94.3-99.8)
	Sub-Total	**132/470 (28.1)**	**130/132 (98.5; 94.6-99.6)**	**331/338 (97.9; 95.8-99.0)**
Residual [2]	**5**	28/148 (18.9)	26/28 (92.9; 77.4-98.0)	117/120 (97.5; 92.9-99.1)
Total		**160/618** [3] **(25.9)**	**156/160 (97.5; 93.7-99.0)**	**448/458 (97.8; 96.0-98.8)**

[1] Additional swabs for use in the Clinical Study were collected at the time of standard of care testing
[2] Leftover, de-identified specimens from standard of care testing
[3] 5 specimens gave inconclusive reference culture results (MALDI-TOF MS log(score) <2.00 by the Direct, Extended Direct and Extraction test methods) and were excluded from the performance calculations

b. Clinical specificity:

Refer to **Section M(3)(a)**, above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

The performance of the ARIES Group A Strep Assay was evaluated in a prospective Clinical Study conducted at four (4) sites in the US (**Section M(3)(a)**). The overall prevalence of *S. pyogenes* (Group A *Streptococcus*) in throat swab specimens was 26.9% (166/618) as determined by the ARIES assay and 25.9% (160/618) as determined by culture. In **Table 10**, the prevalence of *S. pyogenes* as determined by the ARIES assay is stratified by the age and gender of the subjects.

Table 10. Prevalence of *S. pyogenes* positive subjects by age and gender

Age/Gender	Number	ARIES Group A Strep Assay Positive	% Prevalence [1]
<2 years	12	3	25.0
2-11 years	412	134	32.5
12-21 years	162	21	13.0
22-59 years	28	8	28.6
≥60 years	4	0	**0.0**
Male	281	81	28.8
Female	337	85	25.2
Total	**618**	**166**	**26.9**

[1] As determined by the ARIES Group A Strep Assay and excluding specimens in the Clinical Study with inconclusive reference culture results

N. Instrument Name:

ARIES Systems (ARIES System or ARIES M1 System)

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X___ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X___

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X___ or No _____

3. Specimen Identification:

   Specimen identification numbers can be entered manually or using a barcode scanner.

4. Specimen Sampling and Handling:

   An aliquot of the swab transport medium is transferred manually to the ARIES Group A Strep Assay test cassette for automated nucleic acid extraction, PCR amplification/detection and result interpretation.

5. Calibration:

   Calibration is performed by Luminex service personnel using ARIES System Verification Cassettes.

6. Quality Control:

   Refer to **Section M(1)(c)**.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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