Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K191742

B Applicant

Luminex Corporation

C Proprietary and Established Names

ARIES MRSA Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NQX	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a Substantial Equivalence Determination for the ARIES MRSA Assay

B Measurand:

Target DNA sequences for
(1) *mecA* and *mecC* genes for methicillin resistance
(2) *orfX* complex gene of *Staphylococcus aureus*
(3) SCC*mec* junction region

C Type of Test:

Qualitative Real Time Polymerase Chain Reaction (PCR)

III Intended Use/Indications for Use:

A Intended Use(s):

The ARIES MRSA Assay is an integrated, real-time, polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection of methicillin–resistant Staphylococcus aureus (MRSA) DNA from nasal swabs in patients at risk for nasal colonization.

The ARIES MRSA Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings.

The assay is not intended to guide, diagnose, or monitor treatment for MRSA infections. It is not intended to provide results of susceptibility to oxacillin/methicillin. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

The ARIES MRSA Assay is indicated for use with ARIES Systems.

B Indication(s) for Use:

Same as Intended Use

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with ARIES Systems

IV Device/System Characteristics:

A Device Description:

The ARIES MRSA Assay is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test system which consists of the ARIES System or the ARIES M1 System with their included ARIES Software, a sample processing tube, an assay-specific cassette, and an assay-specific protocol file. The ARIES MRSA 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 MRSA Assay cassette directly detects methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs in patients at risk of nasal colonization. Specifically, the ARIES MRSA Assay cassette detects the methicillin resistance genes (*mecA* and *mecC*), *Staphylococcus aureus orfX* gene, the SCC*mec* junction region, and a DNA Sample Processing Control.

Nasal swab specimens are collected using the Liquid Amies Elution Swab (ESwab) Collection and Transport System, or equivalent. A portion of the sample is transferred to the provided 2 mL ARIES MRSA Sample Processing Tube and vortexed. The processed sample is then transferred to the ARIES MRSA Assay cassette.

The specimen is lysed and nucleic acid is extracted using the ARIES System. An extractable sample processing control (SPC) target present in the ARIES MRSA Assay cassette is processed with the specimen. The SPC controls for recovery of extracted nucleic acid, for 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 extracted nucleic acid and SPC are transferred via magnetic beads through the cassette to the ARIES MRSA Assay lyophilized PCR reagents in the PCR tube that contain primer pairs and probes specific to *mecA*/*mecC*, *orfX*, SCC*mec* and the SPC sequence. Each probe is labeled with a distinct fluorophore and detected in a distinct channel of an ARIES System. PCR amplification is performed and assay fluorescence is monitored. Hybridization of a fluorescently labeled probe to the amplified target results in the release of quenching and generation of fluorescence signal that is indicative of PCR generated amplicon. Following amplification, the reaction is heated to separate the fluorescent labeled probe from the amplified target, a process that results in a decrease 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 MRSA Assay protocol and run files. ARIES MRSA Assay results may be reported from the ARIES Software or from the optional SYNCT Software.

B Principle of Operation:

The ARIES MRSA Assay PCR amplification and detection reagents contain primers and probes specific to the methicillin resistance genes (*mecA* and *mecC*), a *Staphylococcus aureus* complex gene (*orf*X), the SCC*mec* region, and the Sample Processing Control (SPC) sequence. Each of the probes are labeled with a distinct fluorophore and detected in distinct channels of the ARIES System. The probes contain a fluorophore on the 5' end of the oligonucleotide sequence and a quencher at the 3' end of the oligonucleotide sequence such that in random coil state when the probe is not hybridized to the target sequence, the fluorescent signal is quenched. PCR amplification is performed and assay fluorescence is monitored. Hybridization of a fluorescently labeled probe to the amplified target results in the release of quenching and generation of fluorescence signal that is indicative of PCR generated amplicon.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Bd Max Mrsa Xt, Bd Max Instrument

B Predicate 510(k) Number(s):
K133605

C Comparison with Predicate(s):

Similarities		
Item	**Device (K191742)**	**Predicate (K133605)**
	ARIES MRSA Assay	**BD Max MRSA XT**
Intended Use	The ARIES MRSA Assay is an integrated, real-time, polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection of methicillin–resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The ARIES MRSA Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. The assay is not intended to guide, diagnose, or monitor treatment for MRSA infections. It is not intended to provide results of susceptibility to oxacillin/methicillin. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing. The ARIES MRSA Assay is indicated for use with ARIES Systems.	The BD MAX MRSA XT assay performed on the BD MAX System is an automated qualitative in vitro diagnostic test for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The test utilizes real-time polymerase chain reaction (PCR) for the amplification of MRSA DNA and fluorogenic target specific hybridization probes for the detection of the amplified DNA. The BD MAX MRSA XT assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. It is not intended to diagnose MRSA infections nor guide or monitor treatment for MRSA infections. A negative result does not preclude nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.
Assay Targets	(1) *mecA* and *mecC* genes for methicillin resistance (2) *orfX* complex gene of *S. aureus* (3) SCC*mec* junction region	(1) SCC*mec*/*orfX* junction area of methicillin-resistant *Staphylococcus aureus* (i.e., MREJ for SCC*mec* Right Extremity Junction). The BD MAX MRSA XT assay is designed to detect MREJ types i, ii, iii, iv, v, vi, vii, ix, xiii, xiv, and xxi; and (2) *mecA* and *mecC* genes for methicillin resistance.
Sample Types	Nasal Swabs	Same
Assay Type	Real-time PCR	Same
Assay Results	Qualitative	Same
Assay Controls	Sample Processing Control (SPC)	Specimen Processing Control (SPC)

Differences		
Item	**Device (K191742)**	**Predicate (K133605)**
	ARIES MRSA Assay	**BD Max MRSA XT**
Extraction Method	Automated by the ARIES Systems	Automated by the BD MAX System
Assay Instrument	ARIES Systems	BD MAX System
Detection	Fluorescent reporter probes for each target, and melting curve analysis	Fluorogenic target-specific hybridization

VI Standards/Guidance Documents Referenced:

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. *Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition*. CLSI document EP37. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – 11th Edition***.** CLSI document M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing – 28th Edition*. CLSI document M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Site-to-Site Reproducibility
The reproducibility of the ARIES MRSA 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 five samples in triplicate: two methicillin-resistant *Staphylococcus aureus* (MRSA) strains each spiked at 1x and 5x Limit of Detection (LoD) in simulated nasal matrix (SNM) in modified Liquid Amies (LA) (spiked SNM+LA) and a negative sample (unspiked SNM+LA) using the same lot of reagents (3 sites X 2 operators X 5 days X 3 replicates = 90 data points per panel member). 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. The reproducibility study results are presented in Table 1.

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

Strain/Level	Positive/Number (%)			
	Site 1	Site 2	Site 3	Overall
MRSA *mecA*+ Moderate Positive 5x LoD[1]	30/30 (100)	30/30 (100)	30/30 (100)	90/90 (100)
MRSA *mecA*+ Low Positive 1x LoD	30/30 (100)	30/30 (100)	30/30 (100)	90/90 (100)
MRSA *mecC*+ Moderate Positive 5x LoD	30/30 (100)	30/30 (100)	30/30[2] (100)	90/90 (100)
MRSA *mecC*+ Low Positive 1x LoD	30/30[2] (100)	30/30 (100)	30/30 (100)	90/90 (100)
Negative	0/30 (0.0)	0/30[3] (0.0)	0/30 (0.0)	0/90 (0.0)

[1]LoD; Limit of detection for MRSA Strains BEI NR-46232 and BAA-2313
5x LoD MRSA *mecA*+ strain BEI NR-46232: 9.75E+04 CFU/mL; 1x LoD MRSA *mecA*+ BEI NR-46232: 1.95E+04 CFU/mL; 5x LoD MRSA strain *mecC*+ ATCC BAA-2313: 3.88E+05 CFU/mL; 1x LoD MRSA *mecC*+ BAA-2313: 7.75E+04 CFU/mL
[2]A single sample was reported as Invalid on initial testing; reported as Positive upon repeat
[3]A single sample was reported as Invalid on initial testing; reported as Negative upon repeat

Within Laboratory Precision/Repeatability
Within laboratory precision/repeatability of the ARIES MRSA 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 precision/repeatability study results are presented in Table 2. The results demonstrated acceptable repeatability and precision from day-to-day with target levels close to the LoD of the assay.

Table 2. Summary of results from the Within Laboratory Precision/Repeatability Study for the AIRES MRSA Assay

Strain/Level	Positive/Tested (%)
MRSA *mecA*+ Moderate Positive 5x LoD[1]	30/30 (100)
MRSA *mecA*+ Low Positive 1x LoD	30/30 (100)
MRSA *mecC*+ Moderate Positive 5x LoD	30/30[2] (100)
MRSA *mecC*+ Low Positive 1x LoD	30/30 (100)
Negative	0/30 (0.0)

[1]LoD; Limit of detection for MRSA Strains BEI NR-46232 and BAA-2313
5x LoD MRSA *mecA*+ strain BEI NR-46232: 9.75E+04 CFU/mL; 1x LoD MRSA *mecA*+ BEI NR-46232: 1.95E+04 CFU/mL; 5x LoD MRSA strain *mecC*+ ATCC BAA-2313: 3.88E+05 CFU/mL; 1x LoD MRSA *mecC*+ BAA-2313: 7.75E+04 CFU/mL
[2]A single sample was reported as Invalid on initial testing; reported as Positive upon repeat

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Cross-reactivity Study
The analytical specificity of the ARIES MRSA Assay was evaluated by testing a panel of 99 organisms that may be found in nasal swab specimens (Table 3). Each organism was tested in triplicate in simulated nasal matrix in Modified Liquid Amies at 10^6 CFU/mL for non-viral organisms, 10^5 TCID50/mL for viruses and 5 μg/mL for human genomic DNA. No false positive were obtained.

Table 3. Organisms tested for potential cross-reaction in the ARIES MRSA Assay

Non-Staphylococcal organisms	
Acinetobacter baumannii	*Listeria monocytogenes*
Acinetobacter haemolyticus	*Legionella pneumophila*
Bacillus cereus	*Moraxella catarrhalis*
Bordetella pertussis	*Micrococcus luteus*
Candida albicans	*Mycoplasma pneumoniae*
Citrobacter freundii	*Mycobacterium tuberculosis* avirulent
Candida glabrata	*Neisseria meningitidis*
Citrobacter koseri	*Listeria monocytogenes*
Chlamydia pneumoniae	*Pasteurella aerogenes*
Corynebacterium bovis	*Pseudomonas aeruginosa*
Corynebacterium flavescens	*Pseudomonas fluorescens*[1]
Corynebacterium genitalium	*Proteus mirabilis*
Cryptococcus neoformans	*Providencia stuartii*
Enterobacter aerogenes	*Proteus vulgaris*
Enterobacter cloacae[1]	*Salmonella enterica subsp. Enterica*
Enterococcus faecalis	*Serratia marcescens*
Enterococcus faecium	*Streptococcus agalactiae*
Escherichia coli (O157:H7)	*Streptococcus anginosus*
Enterococcus flavescens	*Streptococcus mitis*
Enterococcus gallinarum	*Streptococcus mutans*
Enterococcus hirae	*Streptococcus pneumoniae*
Haemophilus influenzae	*Shigella sonnei*
Klebsiella oxytoca	*Streptococcus pyogenes*
Klebsiella pneumoniae (ESBL-producing)	*Streptococcus salivarius*
Klebsiella pneumoniae (KPC-producing)	*Streptococcus sanguinis*
Lactobacillus crispatus	*Streptococcus suis*
	Yersinia enterocolitica
Viruses	
Adenovirus Type 40	Measles virus
Adenovirus Type 7	Mumps virus
Coronavirus 229E	Parainfluenza 1
Coronavirus OC43	Parainfluenza 2
Cytomegalovirus	Parainfluenza 3
Epstein Barr Virus	Rhinovirus type 1A
Influenza A	RSV A
Influenza B	RSV B
Human metapneumovirus	
Coagulase Negative Staphylococci (CoNS)	
Staphylococcus arlettae	*Staphylococcus equorum*
Staphylococcus captis	*Staphylococcus felis*
Staphylococcus carnosus	*Staphylococcus gallinarum*
Staphylococcus chromogenes	*Staphylococcus haemolyticus* Z067
Staphylococcus epidermidis (255-01B)	*Staphylococcus kloosii*
Staphylococcus epidermidis (RP12 CIP 106510)	*Staphylococcus lentus*
Staphylococcus epidermidis (MRSE;RP62A)	*Staphylococcus pulvereri*
Staphylococcus epidermidis (CCF 15990)	*Staphylococcus simulans* Z032
Staphylococcus epidermidis (CCF 16471)	*Staphylococcus warneri* Z113
Coagulase Positive Staphylococci	
Staphylococcus pseudointermedius	*Staphylococcus delphini*

Staphylococcus scheleiferi subsp. Coagulans	*Staphylococcus intermedius*
Staphylococcus scheleiferi subsp. Schleiferi	*Staphylococcus lutrae*
Methicillin-susceptible *Staphylococcus aureus* (MSSA)	
Staphylococcus aureus BAA-1749	*Staphylococcus aureus* 29213
Staphylococcus aureus BAA-1765	*Staphylococcus aureus* 0801675
Staphylococcus aureus BAA-1718	
Other	
Human genomic DNA	

[1]One replicate was reported as Invalid on initial testing; reported as Negative upon repeat

Bioinformatic Analysis

In silico analysis was performed to evaluate the potential for cross-reaction of the ARIES MRSA Assay primers and probes. Non-specific amplification and detection of *Staphylococcus argenteus* by the assay oligos was identified. An investigation into this species indicates high sequence homology between *S. argenteus* and *S. aureus* in the *orfX*, SCC*mec* and *mecA*/*mecC* regions, and that *S. argenteus* is a member of the *S. aureus* complex. Due to the high sequence homology in the *orfX*, SCC*mec* and *mecA*/*mecC* regions between *S. argenteus* and *S. aureus*, it would not be possible for the assay oligos to distinguish between methicillin resistant *S. argenteus* and methicillin resistant *S. aureus*.

Carry-over/Contamination Study

The potential for false-positive results with the ARIES MRSA Assay due to within run or between run cross-contamination was evaluated by testing an alternating series of MRSA "high positive" and negative samples in 20 successive instrument runs using two ARIES instruments. The high positive samples contained MRSA at a concentration of 10^7 CFU/mL in simulated nasal matrix in modified Liquid Amies (SNM+LA). Negative samples comprised SNM+LA alone. One replicate was reported Invalid on initial testing; reported as Positive upon repeat testing. The expected results were obtained for all MRSA positive and negative samples (60/60 each).

Potentially Interfering Substances Study

The potential for interference with the ARIES MRSA Assay was evaluated with endogenous and exogenous substances that may be present in nasal swab specimens (Table 4). Each substance was tested in triplicate in the presence and absence of one strain of *mecA*+ MRSA (NR-46232) and one strain of *mecC*+ MRSA (BAA-2313) at 3x LoD prepared in simulated nasal matrix in Modified Liquid Amies (SNM+LA). No false positives or false negatives were obtained.

Table 4. Substances evaluated for potential interference with the ARIES MRSA Assay

Substance	Test Concentration
Whole Blood	5% (v/v)
Mucin	5 mg/mL
Phenylephrine	0.03 µg/mL
Drixoral (Oxymetazoline)	10% (v/v)
Benzalkonium chloride	0.12%
Propylene glycol	20% (v/v)
Sorbitol[1]	6.45%
Benzyl alcohol[1]	0.5% (v/v)

Hypromellose	0.10%
Phosphoric acid	1.282 mg/mL
Beclomethasone	8.4 µg/mL
Dexamethasone	12 µg/mL
Flunisolide	5 µg/mL
Triamcinolone	22 µg/mL
Budesonide	6.30E-03 µg/mL
Mometasone	4.50E-04 µg/mL
Flonase (Fluticasone)	1.26E-03 µg/mL
ZICAM[2] (Galphimia glauca, Histaminum hydrochloricum)	10% (v/v)
Benzocaine	75 µg/mL
Menthol	0.5 mg/mL
Zanamivir[3]	1 mg/mL
Mupirocin	50 µg/mL
Tobramycin	33 µg/mL
FluMist (Live intranasal influenza virus vaccine)	10% (v/v)

[1]One replicate of SNM+LA negative matrix was reported as Invalid on initial testing; reported as Negative upon repeat

[2]One replicate containing *mecA*+ MRSA strain was reported as Invalid on initial testing; reported as Positive upon repeat

[3]One replicate containing *mecC*+ MRSA strain was reported as Invalid on initial testing; reported as Positive upon repeat

Microbial Interference Study

The potential for interference with the ARIES MRSA Assay by organisms that may be present in nasal swab specimens was investigated using the same list of species that was evaluated for potential cross-reactivity (refer to Table 3). Testing was performed in triplicate with each potentially interfering species in the presence of one strain of *mecA*+ MRSA (NR-46232) or one strain of *mecC*+ MRSA (BAA-2313) at 3x LoD. The potentially interfering species were tested in triplicate in simulated nasal matrix in Modified Liquid Amies at 10^6 CFU/mL for non-viral organisms, 10^5 TCID50/mL for viruses and 5 µg/mL for human genomic DNA. One replicate containing *mecA*+ MRSA was reported as Invalid for *S. gallinarum* on initial testing but was MRSA positive upon repeat testing. One replicate containing *mecC*+ MRSA strain was reported as Invalid for the specimens containing *A. baumannii*, Rhinovirus type 1A and *S. warneri* Z113; however, each specimen was reported as MRSA positive upon repeat testing. These results are acceptable.

Competitive Interference Study

Competitive interference was tested with methicillin-resistant *Staphylococcus aureus* (MRSA) at 1x the limit of detection (LoD) and the co-infecting agent, methicillin-susceptible *Staphylococcus aureus* (MSSA) or methicillin-resistant coagulase-negative Staphylococci (MRCoNS), at increasing concentrations. Each combination was tested in triplicate. The results showed that the ARIES MRSA Assay detected MRSA at 1x LoD in the presence of high concentration of MSSA (~2E+04x LoD) or MRCoNS (~1E+05x LoD). No competitive interference in the ARIES MRSA Assay was observed for co-infections of MRSA with MSSA or MRCoNS.

4. Assay Reportable Range:

Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Specimen Stability
The stability of nasal swabs for use with the ARIES MRSA Assay was evaluated analytically by testing six replicates of each of two methicillin-resistant *Staphylococcus aureus* (MRSA) strains prepared at 3x and 10x Limit of Detection (LoD) in Natural Nasal Matrix (NNM) and stored under different conditions. Unseeded negative sample (NNM only) was included to assess the effect of specimen storage on the performance of the Sample Processing Control (SPC). Specimens stored at 2±2°C were tested at 5 time points over 10 days and specimens stored at 30±1°C were tested at 7 time points over 10 days. The results showed that MRSA specimens stored at both temperatures (2°C and 30°C) generated 100% expected MRSA positive results across all time points tested. An overall cassette invalid rate of 0.53% (4/757) was observed for the testing of MRSA strains and NNM samples in this study. The results of these studies support the stability of nasal swabs for use with the ARIES MRSA Assay when collected using the Liquid Amies Elution Swab (Eswab) Collection and Transport system for up to 10 days at 2-30°C.

Reagent Stability
The shelf-life of the ARIES MRSA 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 19 months from the day of manufacture for the assay cassettes when stored under the recommended conditions.

Cassette open box stability evaluated the performance of the ARIES MRSA Assay Cassettes after removal from the cassette pouch and exposed to ambient temperatures, humidity and light for 10 hours. Over the course of 10 hours, all three lots of cassettes produced expected results, showing that ARIES MRSA Assay Cassettes are stable in ambient temperatures for up to 10 hours after they have been removed from the storage pouch.

Sample Processing Control
Each ARIES MRSA Assay cassette contains a Sample Processing Control (SPC) that is designed to verify 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 methicillin-resistant *Staphylococcus aureus* strain or well characterized methicillin-resistant *Staphylococcus aureus* clinical isolates may be used as positive controls. The ARIES MRSA Assay Kit does not include external positive or negative controls.

External Positive and Negative Controls were tested on a daily basis during the prospective Clinical Study using a total of five ARIES systems and three ARIES MRSA Assay cassette lots. The Positive External Control comprised a standardized suspension of a strain of MRSA at 1.95E+05 CFU/mL (10x LoD). The Negative External Control comprised a standardized suspension of a strain of *S. epidermis* at 4.23E+04 CFU/mL (10x LoD). On initial testing, 182/183 (99.5%) Positive and 178/183 (97.3%) Negative External Controls produced the expected results. Upon repeat testing, all controls produced the expected results.

6. Detection Limit:

The Limit of Detection (LoD) of the ARIES MRSA Assay was estimated for two strains of MRSA by testing various dilutions of enumerated cell stocks in natural nasal matrix (NNM). 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 MRSA *mecA*+ BAA-2312, the LoD was determined to be 1.55×10^4 CFU/mL and for MRSA *mecC*+ NRA-46232, it was 7.75×10^4 CFU/mL.

Inclusivity (Analytical Reactivity)
The inclusivity of the ARIES MRSA Assay was evaluated by testing 55 strains of MRSA in simulated nasal matrix in Modified Liquid Amies (SNM+LA), which include those tested in the LoD Study. All 55 strains produced 3/3 positive results at 3x LoD (2.32×10^5 CFU/mL for *mecC*+ strains BAA-2312 and BAA-2313; 4.65×10^4 CFU/mL for all other strains). Analytical reactivity study results are presented in Table 5.

Table 5. MRSA strains used to evaluate the inclusivity of the ARIES MRSA Assay

Strain Description	Source	Catalog #	Strain/ Location	PFGE Type	SCC*mec* Type
MRSA *mecA*+	BEI	NR-46232 (LoD strain)	NRS703/ Minnesota	USA300	IV
	ATCC	BAA-38	N/A	N/A	I
	ATCC	BAA-1686	N/A	N/A	II
	ATCC	BAA-1687[1]	N/A	N/A	II
	ATCC	BAA-1692	N/A	USA100	II
	ATCC	BAA-1681	N/A	USA100	II
	ATCC	BAA-1682	N/A	USA100	II
	ATCC	BAA-1699	N/A	USA100	II
	BEI	NR-46250	NRS721/ Oregon	USA100	II
	ATCC	BAA-1761	N/A	USA100	II
	ATCC	BAA-1750	N/A	USA200	II
	BEI	NR-46251	NRS722/ Oregon	USA200	II
	ATCC	BAA-39	N/A	N/A	III
	ATCC	BAA-40	N/A	N/A	IIIa
	ATCC	BAA-1717	N/A	USA300	IVa
	ATCC	BAA-1762	N/A	USA300	IVb
	ATCC	BAA-1680	N/A	USA300	IVa
	BEI	NR-46070	NRS384/ Mississippi	USA300	IV
	ATCC	BAA-1683	N/A	USA400	IVa
	ATCC	BAA-1707	N/A	USA400	IV

	ATCC	BAA-1752	N/A	USA400	IV
	ATCC	BAA-1757	N/A	USA400	IV
	ATCC	BAA-1696	N/A	USA400	IVa
	BEI	NR-46207	NRS678/ Connecticut	USA500	IV
	ATCC	BAA-1689	N/A	USA500	IV
	BEI	NR-46220	NRS691/ Georgia	USA500	IV
	ATCC	BAA-1688	N/A	N/A	V
	ATCC	BAA-42	N/A	N/A	VI
	BEI	NR-46177	NRS648/ California	USA600	II
	ATCC	BAA-1751	N/A	USA600	II
	BEI	NR-46218	NRS689/ Georgia	USA700	IV
	ATCC	BAA-1766	N/A	USA700	V
	BEI	NR-46221	NRS692/ Georgia	USA800	IV
	ATCC	BAA-1771	N/A	USA800	IV
	BEI	NR-46197	NRS668/ Colorado	USA800	N/A
	ATCC	BAA-1747	N/A	USA1000	IV
	ATCC	BAA-1769	N/A	USA1000	IV
	ATCC	BAA-1764	N/A	USA1100	IV
	ATCC	BAA-1767	N/A	USA1100	IV
	ATCC	33592	N/A	N/A	III
	ATCC	33593	N/A	N/A	III
	ATCC	43300	N/A	N/A	II
	ATCC	700698	N/A	N/A	II
	ATCC	700699	N/A	N/A	II
	ATCC	700787	N/A	N/A	II
	ATCC	700788	N/A	N/A	II
	ATCC	700789	N/A	N/A	II
	ATCC	BAA-41	N/A	N/A	II
	ATCC	BAA-43	N/A	N/A	IIIa
	ATCC	BAA-44	N/A	N/A	I
	ATCC	BAA-1720	N/A	N/A	II
	ATCC	BAA-2094	N/A	N/A	V
	ATCC	BAA-2096	N/A	N/A	IV
MRSA *mecC+*	ATCC	BAA-2313 (LoD strain)	N/A	N/A	XI
	ATCC	BAA-2312	N/A	N/A	XI

[1]One replicate was reported as Invalid on initial testing; reported as Positive upon repeat

Bioinformatic Analysis

The inclusivity of the ARIES MRSA primers and probes for the targeted regions of the genome was analyzed *in silico* using the Basic Local Alignment Search Tool (BLAST). The region was shown to be well conserved, with nearly 100% of sequences with ≥ 85% identity for *orfX* and *mecA*/*mecC* and approximately 93% of sequences with ≥ 85% identity for SCC*mec* junction. These results are acceptable.

Challenge Study

An additional analytical study was carried out to evaluate the analytical performance of the ARIES MRSA Assay using a panel of challenge strains (Table 6). The challenge panel

included 16 methicillin-resistant *Staphylococcus aureus* (MRSA) strains with high minimum inhibitory concentration (MIC) values of ≥ 16 µg/mL oxacillin and 17 MRSA strains with low MIC values of ≤ 8 µg/mL oxacillin, four borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) strains, 16 empty cassette variants of *Staphylococcus aureus* strains, and one methicillin-resistant *Staphylococcus epidermidis* (MRSE) strain. The strains were tested in triplicate at 3x LoD (MRSA strains) or 10^6 CFU/mL (all other strains). Two strains of MRSA with high MIC values and one strain of MRSA with low MIC value did not generate the expected MRSA positive results when tested at 3x LoD, and were re-tested at 5x LoD and generated the expected MRSA positive results (100% MRSA Positive). The four BORSA strains, the MRSE strain, and all empty cassette variants of *Staphylococcus aureus* generated the expected 0% MRSA positive results (100% MRSA Negative). Analytical challenge study results are presented in Table 6.

Table 6. ARIES MRSA Assay Analytical Challenge Study Results

Target Description	Source	Strain ID	ARIES MRSA Positivity (%)
MRSA with High Oxacillin MIC	ARLG	ARLG-1643	3/3 (100)
	ARLG	ARLG-1644	3/3 (100)
	ARLG	ARLG-1645	3/3 (100)
	ARLG	ARLG-1646	3/3 (100)
	ARLG	ARLG-1662	3/3 (100)
	ARLG	ARLG-1669	0/3[1] (0)
	ARLG	ARLG-1604	3/3 (100)
	ARLG	ARLG-1613	2/3[1] (66.7)
	CDC AR Bank	AR-0215	3/3 (100)
	CDC AR Bank	AR-0218	3/3 (100)
	CDC AR Bank	AR-0219	3/3 (100)
	CDC AR Bank	AR-0220	3/3 (100)
	CDC AR Bank	AR-0223	3/3 (100)
	CDC AR Bank	AR-0224	3/3 (100)
	CDC AR Bank	AR-0227	3/3 (100)
	CDC AR Bank	AR-0228	3/3 (100)
MRSA with Low Oxacillin MIC	ARLG	ARLG-1642	2/3[1] (66.7)
	Lyon University	20130237	3/3 (100)
	Lyon University	20130524	3/3 (100)
	CDC AR Bank	AR-0216	3/3 (100)
	CDC AR Bank	AR-0217	3/3 (100)
	CDC AR Bank	AR-0221	3/3 (100)
	CDC AR Bank	AR-0225	3/3 (100)
	CDC AR Bank	AR-0226	3/3 (100)
	ATCC	BAA-1688	3/3 (100)
	CDC AR Bank	AR-472	3/3 (100)
	CDC AR Bank	AR-473	3/3 (100)
	CDC AR Bank	AR-474	3/3 (100)
	CDC AR Bank	AR-475	3/3 (100)
	CDC AR Bank	AR-476	3/3 (100)
	CDC AR Bank	AR-477	3/3 (100)
	CDC AR Bank	AR-478	3/3 (100)
	CDC AR Bank	AR-479	3/3 (100)
BORSA	CDC AR Bank	AR-0489	0/3 (0)
	CDC AR Bank	AR-0490	0/3 (0)
	CDC AR Bank	AR-0491	0/3 (0)
	CDC AR Bank	AR-0492	0/3 (0)
	Lyon University	20101270	0/3 (0)

Empty Cassette Variant of SA	Lyon University	20112896	0/3 (0)
	Lyon University	20112911	0/3 (0)
	Lyon University	20120556	0/3 (0)
	Lyon University	20120844	0/3 (0)
	Lyon University	20120871	0/3[2] (0)
	Lyon University	20120984	0/3 (0)
	Lyon University	20121469	0/3 (0)
	Lyon University	20121544	0/3[2] (0)
	Lyon University	20121635	0/3 (0)
	Lyon University	20121891	0/3 (0)
	Lyon University	20130769	0/3 (0)
	Lyon University	20131190	0/3 (0)
	Lyon University	20131273	0/3 (0)
	Lyon University	20131727	0/3 (0)
	Lyon University	20140852	0/3 (0)
MRSE	ATCC	51625	0/3 (0)

[1]Repeat testing at 5x LoD reported as 3/3 (100%) MRSA Positive
[2]One replicate was reported as Invalid on initial testing; reported as Negative upon repeat

7. Assay Cut-Off:

For the ARIES MRSA Assay, each target (*mecA/mecC*, *orfX*, and SCC*mec*) has a Ct cut-off, T_m window, and T_m Peak Threshold. In addition, the internal sample processing control (SPC) also has a corresponding Ct cut-off, T_m window, and T_m Peak Threshold. Collectively, the cut-off values compose the assay protocol file parameters, which are used to determine the assay result for the detection of the target as Positive, Negative, or Invalid. These values are hard-coded into the ARIES MRSA Assay Protocol File and are not modifiable. The Assay Protocol File parameters were determined, and their performance in the ARIES MRSA Assay were evaluated according to the following general procedure:

- Initial Assay Protocol File parameters were set during internal optimization and benchmarking studies.
- The final Assay Protocol File parameters were then established during internal verification studies using data from optimization, benchmarking and verification.
- 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 MRSA Assay.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable.

2. Matrix Comparison:

Comparison of Performance with Natural and Simulated Matrices
To provide a sufficient quantity of material for testing, a simulated nasal 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 MRSA Assay cassette.

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 using MRSA strain NR-46232. The results demonstrated similar analytical sensitivity in both matrices and there were negligible differences in LoD and MRSA targets. The study therefore provided acceptable evidence to support the use of simulated matrix in the Analytical Studies to characterize the performance of the ARIES MRSA Assay.

Nasal Swab Comparison Study
A nasal swab equivalency study was performed to evaluate the reproducibility of the ARIES MRSA Assay with two different nasal swab types, Regular Nylon Flocked Swab (Copan Catalog Number: 480C) and Flexible Minitip Nylon Flocked Swab (Copan Catalog Number: 482C). The swabs were evaluated using one strain of methicillin-resistant *Staphylococcus aureus* (MRSA) *mecA*+ (NR-46232) at three concentrations, as well as a negative sample (Simulated nasal matrix in Modified Liquid Amies, SNM+LA). Samples at intermediate concentrations prepared in SNM+LA were transferred to Modified Liquid Amies using each of the two nasal swab types to reach the final testing concentrations at 3x LoD, 5x LoD and 10x LoD, respectively, and then tested on the ARIES MRSA Assay. The test results demonstrated that both swab types generated 100% expected MRSA positivity for each strain of the concentrations tested. Both swab types also generated 0% positivity (100% negativity) for negative samples. Swab equivalency study results are presented in Table 7.

Table 7. ARIES MRSA Assay Nasal Swab Equivalency Results

Assay Target	Part Number	Test Concentration (CFU/mL)	MRSA Positivity (%)	
			Regular swab (Copan 480C)	Flexible mini-tip swab (Copan 482C)
MRSA *mecA*+	NR-46232	3x LoD (5.85E+04)	6/6 (100)	6/6 (100)
		5x LoD (9.75E+04)	6/6 (100)	6/6 (100)
		10x LoD (1.95E+05)	6/6 (100)	6/6 (100)
Negative (SNM+ LA)	N/A	N/A	0/6 (0)	0/6 (0)

C Clinical Studies:

1. Clinical Sensitivity:
Clinical performance of the ARIES MRSA Assay for nasal swab specimens collected from patients at risk for methicillin-resistant *Staphylococcus aureus* (MRSA) colonization was established through a clinical study.

Performance of the ARIES MRSA Assay was evaluated prospectively from August 2018 to February 2019 at four (4) geographically distinct clinical sites within the United States using the ARIES System. Specimens included in the clinical study consisted of excess leftover de-identified, nasal clinical specimens collected using the Liquid Amies Elution Swab (Eswab) Collection and Transport system, or equivalent, from patients at risk for nasal colonization. All eligible clinical specimens were tested by both the reference method (direct and enriched bacterial culture) and ARIES MRSA Assay and the results compared. Reference method

testing was performed at a centralized testing facility while ARIES MRSA Assay testing was performed at each clinical site on their own clinical specimens.

A total of 2254 nasal swab specimens from subjects at risk for MRSA nasal colonization were collected. Of these 2,254 specimens, 472 were excluded from the study based on inclusion/exclusion criteria leaving a total of 1,782 unique specimens that met the pre-determined eligibility criteria for inclusion in the study.

In total, 1,782 specimens were enrolled in the study and tested for methicillin-resistant *Staphylococcus aureus* by both the reference method and the ARIES MRSA Assay. There were 20 specimens that when tested with ARIES MRSA Assay yielded an invalid result due to run failure or instrument error giving an invalid rate of 1.1% (20/1,782). None of these specimens were re-tested due to insufficient specimen volume.

For the 1,762 eligible specimens that were included in the device performance calculations, clinical sensitivity of the ARIES MRSA Assay against direct and enriched bacterial culture (Table 8), was 93.3% (97/104) with a lower bound 95% confidence interval of 87%. Clinical specificity of the ARIES MRSA Assay was 93.5% (1550/1658) with a lower bound 95% confidence interval of 92%. Of the 108 specimens that were MRSA-negative by culture but MRSA-positive by the ARIES MRSA Assay, culture showed that 63 specimens were *S. aureus* and 45 were negative (no growth).

When ARIES MRSA Assay was compared to Direct Culture only (Table 9), the positive percent agreement of the ARIES MRSA Assay was 93.5% (87/103) with a lower bound 95% confidence interval of 87%. Negative percent agreement of the ARIES MRSA Assay was 92.9% (1550/1658) with a lower bound 95% confidence interval of 92%. Overall, performance was determined to be acceptable.

Table 8: ARIES MRSA Assay Performance Compared to Direct and Enriched Culture

ARIES MRSA Assay	Direct and Enriched Bacterial Culture for MRSA		
	Positive	Negative	TOTAL
Positive	97	108	205
Negative	7	1550	1557
TOTAL	104	1658	1762
Sensitivity (95% CI)	93.3% (87% - 97%)		
Specificity (95% CI)	93.5% (92% - 95%)		

Table 9: ARIES MRSA Assay Performance Compared to Direct Culture (Informational Only)

ARIES MRSA Assay	Direct Bacterial Culture for MRSA		
	Positive	Negative	TOTAL
Positive	87	118	205
Negative	6	1551	1557
TOTAL	93	1669	1762
Positive Percent Agreement (95% CI)	93.5% (87% - 97%)		
Negative Percent Agreement (95% CI)	92.9% (92% - 94%)		

2. Clinical Specificity:

   See Clinical Sensitivity above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The performance of the ARIES MRSA Assay was evaluated in a prospective Clinical Study conducted at four (4) sites in the US. The overall prevalence of MRSA in nasal swab specimens was 5.9% (104/1762) as determined by culture (direct plus enriched) and 11.6% (205/1762) as determined by the ARIES assay. In Table 10, the prevalence of MRSA as determined by the ARIES assay is stratified by the age and gender of the subjects.

Table 10. Prevalence of MRSA positive subjects stratified by age and gender

	Number of Subjects	ARIES MRSA Assay Positive	% Prevalence[1]
Gender			
Male	954	122	12.8
Female	808	83	10.3
Overall	1762	205	11.6
Age (yrs)			
<2	405	21	5.2
2 – 11	251	21	8.4
12 – 21	189	19	10.1
22 – 59	481	74	15.4
≥60	436	70	16.1
Overall	1762	205	11.6

[1]As determined by the ARIES MRSA Assay

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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