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

A. 510(k) Number:

K162772

B. Purpose for Submission:

To obtain a substantial equivalence determination for the ARIES® GBS Assay

C. Measurand:

A conserved genomic region of *Streptococcus agalactiae* (Group B Streptococcus, GBS)

D. Type of Test:

Real-time polymerase chain reaction (PCR) test

E. Applicant:

Luminex Corporation

F. Proprietary and Established Names:

Aries GBS Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3740: *Streptococcal* spp. serological reagents

2. Classification:

   Class I

3. Product code:

   NJR: Nucleic Acid Amplification Assay System, Group B Streptococcus, Direct
   OOI: Real-Time Nucleic Acid Amplification System

4. Panel:

   83: Microbiology

H. Intended Use:

1. Intended use(s):

   The ARIES GBS Assay, performed on ARIES Systems, is a real-time polymerase chain reaction (RT-PCR) based qualitative in vitro diagnostic test. The ARIES GBS Assay is designed to detect Group B Streptococcus (GBS) nucleic acid from 18-24 hour Lim broth enrichments of vaginal-rectal specimen swabs obtained from pregnant women.

   The ARIES GBS Assay is intended for use as a method for detection of GBS colonization in antepartum women. It is not intended to diagnose or monitor treatment of a GBS infection.

   The ARIES GBS Assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

2. Indication(s) for use:

   Same as the Intended Use.

3. Special conditions for use statement(s):

   This assay has been validated with Lim broth medium only. Performance of the assay has not been validated with other GBS selective broth enrichment media.

   For *in vitro* diagnostic use only.

   For prescription use only.

4. Special instrument requirements:

   For use with the ARIES System or ARIES M1 System.

   Note: The ARIES System and ARIES M1 System are collectively referred to as the ARIES Systems throughout this memo.

I. Device Description:

The ARIES GBS Assay is an automated *in vitro* diagnostic test for the qualitative detection of Group B Streptococcus (GBS, *Streptococcus agalactiae*) DNA from Lim broth enrichments from vaginal/rectal swab specimens obtained from antepartum pregnant women.

The ARIES GBS Assay is a real-time polymerase chain reaction (RT-PCR)-based test system comprised of either the ARIES System or the ARIES M1 System (and associated software), the ARIES GBS Assay cassette, and an assay-specific protocol file. The ARIES GBS 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 assay on one sample.

Vaginal/rectal swab specimens are transported to the laboratory and enriched overnight in Lim broth, per CDC guidelines. Once the Lim broth enrichment is added to the assay cassette, the ARIES GBS Assay automates the process from nucleic acid extraction to specimen result. The ARIES GBS Assay cassette contains an internal extractable sample processing control (SPC) target that is processed with the specimen. The SPC controls for specimen lysis, for recovery of extracted nucleic acid, for the presence of inhibitory substances, and for PCR reagent and instrument integrity. The cycle threshold (Ct) value of the SPC is designed to verify proper specimen lysis and nucleic acid extraction, to identify PCR inhibition, if any, and verify proper function of the extraction system and real-time instrument. The melting temperature (Tm) value of the SPC is used as a reference for determining the target Tm.

The extracted nucleic acid and SPC are transferred via magnetic beads through the cassette to the ARIES GBS Assay lyophilized PCR reagents in the PCR tube that contain primers specific to GBS and the SPC sequence. The specific primer pairs are labeled with distinct fluorophore labels. PCR amplification is performed and assay fluorescence is monitored on the ARIES Systems. The instrument fluorescence output is analyzed and test results are determined using the ARIES GBS Assay Protocol File. ARIES GBS Assay results may be reported from the ARIES Software or from the optional SYNCT Software.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Xpert GBS LB Assay

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

   K121539

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	ARIES GBS Assay (K162772)	**Xpert GBS LB Assay (K121539)**
Intended Use	The ARIES GBS Assay, performed on ARIES Systems, is a real-time polymerase chain	The Cepheid Xpert GBS LB Assay, performed on the GeneXpert Instrument Systems, is

Similarities		
Item	**Device**	**Predicate**
	ARIES GBS Assay (K162772)	**Xpert GBS LB Assay (K121539)**
	reaction (RT-PCR) based qualitative in vitro diagnostic test. The ARIES GBS Assay is designed to detect Group B Streptococcus (GBS) nucleic acid from 18-24 hour Lim broth enrichments of vaginal-rectal specimen swabs obtained from pregnant women. The ARIES GBS Assay is intended for use as a method for detection of GBS colonization in antepartum women. It is not intended to diagnose or monitor treatment of a GBS infection. The ARIES GBS Assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.	a qualitative *in vitro* diagnostic test designed to detect Group B *Streptococcus* (GBS) DNA from enriched vaginal/rectal swab specimens, using fully automated realtime polymerase chain reaction (PCR) with fluorogenic detection of the amplified DNA. Xpert GBS LB Assay testing is indicated as an aid in determining GBS colonization status in antepartum women. The Xpert GBS LB Assay is used for antepartum testing on enriched Lim broth cultures of vaginal/rectal swabs after 18-24 hours of incubation. The Xpert GBS LB assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin allergic women.
Analyte	Conserved region of the GBS genome	Same
Sample type	Vaginal/rectal swab enriched in Lim broth	Same
Assay format	Real-time PCR	Same
Assay results	Qualitative	Same
Single-use test	Disposable, single-use cartridge	Same
Sample extraction	Automated by the instrument system	Same

Differences		
Item	**Device**	**Predicate**
	ARIES GBS Assay (K162772)	**Xpert GBS LB Assay (K121539)**
Internal assay controls	Sample Processing control	Sample Processing Control; Internal control; Probe Check
Instruments	ARIES System, ARIES M1 System	Cepheid GeneXpert Dx System, GeneXpert Infinity-48 System, GeneXpert Infinity-80 System
Processing Cartridge and Reagents	ARIES GBS Assay Cartridge	GeneXpert GBS assay cartridge

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

Not applicable.

L. Test Principle:

The Lim broth enriched specimen is vortexed and then pipetted into the ARIES GBS Assay cassette sample chamber. The cassette is then placed into an ARIES magazine which can hold up to six cassettes. The magazine is inserted into an ARIES instrument. A barcode on top of the ARIES GBS Assay cassette is automatically scanned by the ARIES instrument, associating a preloaded ARIES GBS Assay Protocol File with the cassette. The ARIES GBS Assay Protocol File contains the necessary parameters to run the cassette, analyze data, and generate reports.

Once a run is started, the sample processing control (SPC) is automatically added to the sample chamber of the cassette to control for sample lysis, recovery of extracted nucleic acid, detection of inhibitory substances, and confirmation of PCR reagent integrity. Sample and SPC lysis, as well as isolation and purification of nucleic acids, are automated within the ARIES Systems and the ARIES GBS Assay cassette. Purified nucleic acids are automatically transferred to the cassette's PCR tube that contains the lyophilized GBS Master Mix for the PCR amplification step. The lyophilized GBS Master Mix contains an assay-specific master mix capable of performing the designated assay on one sample.

During PCR amplification, a quencher-modified 2'-deoxyisoguanosine triphosphate (iGTP) is incorporated by the polymerase opposite an 2'-deoxy-5-methyl-isocytidine (iC) and a fluorophore reporter attached to a PCR primer. If the 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 Systems instrumentation.

Following real-time 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 (Tm) of the amplicon.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility Study
Between-laboratory reproducibility for the ARIES GBS Assay was evaluated using the following panel prepared by spiking cultured GBS into Lim broth at four concentration levels:

1) Negative (N)
2) High Negative (HN), 0.8 x LoD
3) Low Positive (LP), 1.5 x LoD
4) High Positive (HP), 100.0 x LoD

Three replicates of each panel member and the controls were processed and tested with the ARIES GBS Assay at each site by two operators for five non-consecutive days using three instruments (2 operators x 3 replicates x 5 days x 3 sites = 90 results per concentration for each analyte). The results are shown in the **Table 1**.

Table 1: ARIES GBS Assay Inter-Laboratory Reproducibility

Panel ID	Site 1				Site 2				Site 3				Combined		
	Detected Pos/Total	% Pos	Avg Ct	Ct %CV	Detected Pos/Total	% Pos	Avg Ct	Ct %CV	Detected Pos/Total	% Pos	Avg Ct	Ct %CV	Detected Pos/Total	% Pos	Avg Ct
N	0/30	0.0	N/A*	4.2	0/30	0.0	N/A*	4.1	0/30	0.0	N/A*	4.7%	0/90	0.0	N/A*
HN	18/30	60.0	38.4	1.8	11/30	36.7	38.7	1.6	17/30	56.7	38.5	1.5%	46/90	51.1	38.5
LP	26/30	86.7	37.6	2.3	29/30	96.7	37.2	1.5	26/30	86.7	37.8	1.6%	81/90	90.0	37.5
HP	30/30	100	29.8	1.3	30/30	100	29.9	1.0	30/30	100	29.9	0.9%	90/90	100	29.9

*N/A = Not Applicable

These results met the pre-defined acceptance criteria for the various panels and are described in labeling.

Precision Study
Within-laboratory, inter-lot and inter-instrument precision was evaluated for the ARIES GBS Assay using the same panel of contrived specimens as in the reproducibility study above.

The study was conducted using three lots and three instruments over 12 non-consecutive days at one site (in-house) with two operators (2 operators x 2 times per

day x 12 days x 2 replicates = 96 results per panel member). One operator used one instrument and the other used two instruments. The results are shown in **Table 2**.

Table 2: ARIES GBS Assay Intra-Laboratory Precision

Analyte	Panel ID	Operator 1			Operator 2			Combined		
		Detected Pos/Total	% Pos	Average Ct	Detected Pos/Total	% Pos	Average Ct	Detected Pos/Total	% Pos	Average Ct
GBS (CDC 2008232729)	N	0/48	0.0%	N/A*	0/48	0.0%	N/A*	0/96	0.0%	N/A*
	HN	37/48	77.1%	38.2	38/48	79.2%	38.0	75/96	78.1%	38.1
	LP	45/48	93.8%	37.5	47/48	97.9%	37.3	92/96	95.8%	37.4
	HP	48/48	100.0%	30.0	48/48	100.0%	29.8	96/96	100.0%	29.9

*N/A = Not Applicable

These results met the pre-defined acceptance criteria for the various panels and are described in labeling.

b. Linearity/assay reportable range:

Not applicable.

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

Process Control:
Each ARIES assay cassette contains a Sample Process Control (SPC), which is processed with the sample and analyzed during the amplification reaction. The SPC verifies sample lysis, nucleic acid extraction, and proper reagent, cassette, ARIES instrument, and assay protocol performance. The SPC has a known Tm 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 GBS strain or well characterized GBS clinical isolates may be used as positive controls. Lim broth may be used as a negative control. The ARIES GBS Assay cassette kit does not include external positive and negative controls.

Specimen Stability:
Fresh specimen stability was determined using samples consisting of cultured GBS spiked into Lim broth at four concentration levels including moderate positive, low positive, high negative, and negative concentrations. The samples were stored at both ambient (18 °C to 25 °C) and refrigerated (2 °C to 8 °C) temperatures and tested in triplicate at multiple time points using the ARIES GBS Assay. Specifically, the samples stored at ambient temperature (18 °C to 25 °C) were evaluated for stability up to 24 hours, with testing at multiple intervals between 0 and 24 hours plus an

additional time point past 24 hours. The samples stored refrigerated (2 °C to 8 °C) were evaluated for stability up to 7 days, with testing at multiple intervals between 0 and 7 days plus an additional time point past 7 days. The study results showed that enriched specimens for the ARIES GBS Assay are stable for up to 24 hours when stored at 18 °C to 25 °C and up to 7 days when stored at 2 °C to 8 °C.

Frozen specimen stability was determined using samples consisting of cultured GBS spiked into Lim broth at four concentration levels including moderate positive, low positive, high negative, and negative concentrations. The samples were stored at -65 °C to -95 °C and tested in triplicate at multiple time points using the ARIES GBS Assay. Data was collected and evaluated for frozen stability based on testing at multiple intervals between 0 and 3 months plus an additional time point past 3 months. Based on the results, enriched specimens for the ARIES GBS Assay are stable for up to 3 months when stored at -65 °C to -95 °C.

Lim Broth Stability:
A study was performed to evaluate the performance of the ARIES GBS Assay across the range of Lim broth incubation time. The study tested a panel of contrived samples consisting of Lim broth spiked with varying amounts of GBS bacteria measured in CFU/mL for positive samples and blank Lim broth as negative samples. The samples were incubated at 35 °C to 37 °C and tested in triplicate at multiple intervals between 18 hours and 24 hours plus an additional time point past 24hours. Based on the study results, samples can be enriched in Lim broth at 35 °C to 37 °C from 18 hours to 24 hours with no difference in performance of the ARIES GBS Assay.

Shelf-Life Stability:
A real time stability study was performed to evaluate the shelf life of ARIES GBS cassettes. Stability was assessed by testing 6 replicates of GBS (GBS spiked into Lim broth) and negative targets (Lim broth) on three different lots of ARIES GBS cassettes stored at 2 different temperatures, refrigerated (2 °C to 8 °C) and room temperature (15 °C to 30 °C), at 10 different time points extending out to 19 months. The study results support the stability of the ARIES GBS cassettes for up to 11 months when stored either refrigerated (2 °C to 8 °C) or at room temperature (15 °C to 30 °C).

d. Detection limit:

A Limit of Detection (LoD) study was performed to evaluate the analytical sensitivity of the ARIES GBS Assay using five common GBS serotypes (Ia, Ib, II, III, V, and one non-hemolytic serotype). Preliminary LoD concentrations were determined using serial dilutions of each quantified GBS serotype in Lim broth. These preliminary LoD concentrations were confirmed by testing 20 replicates of each strain. All GBS serotype concentrations were verified by colony counting (CFU/ml). The LoD was defined as the point at which at least 95% of all replicates

tested positive (C95). The final LoD concentrations for the tested GBS serotypes are shown in **Table 3**.

Table 3: ARIES GBS Assay LoD			
Strain #	**GBS Serotype**	**GBS Strain**	**Concentration Detected (CFU/ml)**
1	I a	ATCC BAA -1138	5.9×10^3
2	I b	CDC 2008232729	1.7×10^3
4	II	ATCC BAA -1175	1.4×10^4
5	III	ATCC BAA -1176	6.7×10^3
6	V	CDC 2008232731	4.9×10^3
7	Non-Hemolytic II	ATCC BAA -13813	8.2×10^2

These LoD values are described in the labeling and they were used in determining the spiking levels for all subsequent analytical studies.

e. *Analytical reactivity:*

The analytical reactivity (inclusivity) of the ARIES GBS Assay was evaluated against 12 GBS serotypes (one strain per serotype), which differed from the strains tested in the LoD study. Each isolate listed in **Table 4** was diluted in Lim broth to a concentration near the LoD and tested in triplicate with the ARIES GBS Assay.

Table 4: ARIES GBS Assay Inclusivity			
Strain #	**GBS Serotype**	**GBS Strain**	**Concentration Detected (CFU/ml)**
1	I a	CDC#2008232728	1.1×10^4
2	I b	ATCC BAA-1174	2.4×10^4
3	I c	ATCC 27591	7.6×10^3
4	II	CDC#2008232738	2.2×10^4
5	III	CDC#2008232582	4.0×10^3
6	IV	CDC#2011201884	2.3×10^4
7	V	ATCC BAA 611	5.0×10^3
8	VI	CDC#2010228816	4.5×10^3
9	VII	CDC#4832-06	3.8×10^3
10	VIII	CDC#5030-08	2.2×10^4
11	IX	CDC#7509-07	1.4×10^4
12	Non-Hemolytic II	CDC#2009207634	2.2×10^4

All GBS strains were identified as positive by the ARIES GBS Assay. These study results are acceptable and they are described in the labeling.

f. Analytical specificity:

i. Microbial cross-reactivity

A study was performed to evaluate the performance of the ARIES GBS Assay in the presence of 113 potentially cross-reacting bacteria, yeast and viruses commonly found in vaginal/rectal swab specimens. Clinically relevant levels of bacteria/yeast ($\geq 10^6$ CFU/mL) and viruses ($\geq 10^5$ $TCID_{50}$/mL or highest available concentration) were spiked into negative Lim broth and tested in triplicate on the ARIES System. The panel of strains included in the cross-reactivity study is shown in **Table 5**.

Table 5: ARIES GBS Assay Cross-Reactivity Study Species List

Bacteria/Fungi	*Haemophilus ducreyi*	*Salmonella typhimurium*
Achromobacter xerosis	*Haemophilus influenza*	*Serratia marcescens*
Acinetobacter calcoaceticus	*Helicobacter pylori*	*Shigella flexneri*
Acinetobacter lwoffi	*Kingella denitrificans*	*Shigella sonnei*
Aerococcus viridans	*Kingella kingae*	*Staphylococcus aureus*
Aeromonas hydrophila	*Klebsiella oxytoca*	*Staphylococcus epidermidis*
Alcaligenes faecalis	*Klebsiella pneumonia*	*Staphylococcus saprophyticus*
Arcanobacterium pyogenes	*Lactobacillus acidophilus*	*Streptococcus anginosus*
Bacillus cereus	*Lactobacillus brevis*	*Streptococcus bovis*
Bacillus subtilis	*Lactobacillus casei*	*Streptococcus canis*
Bacteroides fragilis	*Lactobacillus delbrueckii lactis*	*Streptococcus dysgalactiae*
Bifidobacterium adolescentis	*Lactobacillus jensenii*	*Streptococcus mitis*
Bifidobacterium breve	*Listeria monocytogenes*	*Streptococcus mutans*
Brevibacterium linens	*Micrococcus luteus*	*Streptococcus oralis*
Campylobacter jejuni	*Mobiluncus mulieris*	*Streptococcus parasanguinis*
Candida albicans	*Moraxella lacunata*	*Streptococcus pneumoniae*
Candida dubliniensis	*Moraxella osloensis*	*Streptococcus pyogenes*
Candida glabrata	*Morganella morganii*	*Streptococcus salivarius*
Candida parapsilosis	*Mycobacterium gordonae*	*Streptococcus sanguinis*
Candida tropicalis	*Mycobacterium smegmatis*	*Streptococcus suis*
Chlamydia trachomatis	*Mycoplasma genitalium*	*Streptococcus uberis*
Chromobacterium violaceum	*Neisseria gonorrhoeae*	*Streptomyces griseus*
Citrobacter freundii	*Peptococcus* spp.	*Treponema pallidum*
Clostridium difficile	*Peptostreptococcus anaerobius*	*Trichomonas vaginalis*
Clostridium sporogenes	*Plesiomonas shigelloides*[3]	*Ureaplasma urealyticum*
Corynebacterium spp.[2]	*Porphyromonas asaccharolytica*	*Veillonella parvula*
Corynebacterium urealyticum	*Prevotella melaninogenica*	*Vibrio parahaemolyticus*
Corynebacterium xerosis	*Propionibacterium acnes*	*Weissella paramesenteroides*

Cryptococcus neoformans	*Proteus mirabilis*	*Yersinia enterocolitica*
Elizabethkingia meningoseptica[1]	*Proteus vulgaris*	**Viruses**
Enterobacter aerogenes	*Pseudomonas aeruginosa*	Cytomegalovirus (CMV)
Enterobacter cloacae	*Pseudomonas fluorescens*	Hepatitis B virus (HBV)
Enterococcus durans	*Rahnella aquatilis*	Hepatitis C virus (HCV)
Enterococcus faecalis	*Rhizobium radiobacter*	Herpes Simplex Virus, type I (HSV1)
Enterococcus faecium	*Rhodospirillum rubrum*	Herpes Simplex Virus, type II (HSV2)
Escherichia coli	*Ruminococcus productus*	Human immunodeficiency virus (HIV-1)
Flavobacterium spp.	*Saccharomyces cerevisiae*	Human Papilloma Virus 16 (HPV 16)
Gardnerella vaginalis	*Salmonella enterica*[4]	Human Papilloma Virus 18 (HPV 18)

[1] Formerly *Chryseobacterium meningosepticum*
[2] Formerly *Corynebacterium genitalium*
[3] Formerly *Plesiomonas* spp.
[4] Formerly *Salmonella choleraesuis*

None of the 113 bacteria , yeast or viruses that were tested cross-reacted with the ARIES GBS Assay. These study results are acceptable and they are described in the labeling.

ii. Microbial interference

Microbial interference studies were performed in triplicate with the same panel of 113 potentially interfering bacteria, yeast and viruses that was tested in the microbial cross-reactivity study. The panel of strains included in the microbial interference study is shown in **Table 5** above. Clinically relevant levels of bacteria/yeast ($\geq 10^6$ CFU/mL) and viruses ($\geq 10^5$ $TCID_{50}$/mL or highest available concentration) and GBS near the LoD were spiked into Lim broth and tested in triplicate on the ARIES System. None of the bacteria, yeast or viruses demonstrated interference with the detection of the Group A Streptococcal strains in any of the replicates tested.

iii. Interfering substances

Twenty eight chemical and biological substances were evaluated for the potential to interfere with the ARIES GBS Assay, including human whole blood (2.0% v/v), human feces (0.47% w/v), human urine (2.0% v/v), and human genomic DNA (1.2×10^6). Each substance was spiked into Lim broth with GBS near the LoD and tested in triplicate on the ARIES System. None of the substances tested were found to interfere with the ARIES GBS Assay in any of the replicates tested. These study results are acceptable and they are described in the labeling.

iv. Carryover/Cross-contamination

A study to assess carryover contamination was conducted for the ARIES GBS Assay using 30 high positive GBS samples (at 100 x LoD) in series alternating with 30 GBS negative (Lim broth) samples. The high positive samples were run

adjacent to negative samples across ten consecutive runs using one ARIES System. No carry-over or cross contamination was observed in the course of this study. These study results are acceptable and they are described in the labeling.

g. Assay cut-off:

The assay cut-off values are hard-coded into the ARIES GBS Assay protocol file and are not modifiable. These values were established through Receiver Operator Characteristic (ROC) analysis using data collected during LoD testing and internal clinical specimen testing with a sample set. Vaginal/rectal swab clinical specimens from antepartum women were enriched in LIM broth and tested with three lots of ARIES GBS Assay cassettes and four instruments.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

A comparison study was conducted between negative clinical matrix and a contrived negative matrix in order to validate the use of Lim broth in place of a natural negative clinical matrix (pooled negative clinical specimens enriched in Lim broth at 37 ^{o}C, 5% CO_2 for 18 to 24 hours) for use in the analytical studies. Each matrix type was spiked with GBS at the same four concentrations used in the reproducibility studies in section **M**.1.*a* above. The matrix comparison study results are shown in **Table 6**.

Table 6: ARIES GBS Assay Matrix Comparison Study

Panel ID		Contrived Negative Matrix		Pooled Negative Clinical Matrix	
		Detected	% Pos	Detected	% Pos
GBS (CDC 2008232729)	N	0/18	0.0%	0/18	0.0%
	HN	5/18	27.8%	6/18	33.3%
	LP	17/18	94.4%	18/18	100.0%
	HP	18/18	100.0%	18/18	100.0%

These studies demonstrate that the contrived negative matrix is equivalent to a clinical matrix. These study results are acceptable and support the use of contrived matrix in analytical studies.

c. Fresh versus frozen comparison :

Fresh contrived specimens and frozen specimens were prepared as described in the reproducibility studies (**M**.1.*a*) above. The results of the fresh versus frozen study are

shown in **Table 7**.

Table 7: ARIES GBS Assay Fresh Versus Frozen Study

Panel ID		Fresh Specimens Stored at 18 °C to 25 °C		Fresh Specimens Stored at 2 °C to 8 °C		Fresh Specimens Stored at -65 °C to -95 °C	
		Detected	% Pos	Detected	% Pos	Detected	% Pos
GBS (CDC 2008232729)	N	0/15	0.0%	0/15	0.0%	0/18	0.0%
	HN	14/15	93.3%	9/15	60.0%	6/18	33.3%
	LP	15/15	100.0%	15/15	100.0%	27/27	100.0%
	HP	15/15	100.0%	15/15	100.0%	18/18	100.0%

These studies demonstrate that freezing does not appear to impact results for the ARIES GBS Assay. These study results are acceptable and support the use of frozen specimens in the clinical studies.

3. Clinical studies:

a. Clinical Sensitivity:

Performance of the ARIES GBS Assay was established during a prospective study conducted from May, 2016 to August, 2016. Six hundred eighty-eight unique specimens that met the predetermined inclusion criteria were enrolled in the study and tested for GBS by both the reference culture and the ARIES GBS Assay at three geographically distinct clinical sites within the United States.

Specimens for the clinical study consisted of leftover, de-identified Lim broth cultures from pregnant women at 35 – 37 weeks of gestation (antepartum) whose standard of care screening called for the collection of vaginal-rectal swab specimens for GBS testing. The vaginal-rectal swab specimens were enriched in Lim broth for 18-24 hours at 35-37°C. After enrichment, an aliquot of the Lim broth was used for standard-of-care testing, and the leftover Lim broth was used for reference culture testing and for ARIES GBS Assay testing. Reference culture testing was performed in accordance with published CDC guidelines whereby enriched Lim broth aliquots were subcultured to 5% sheep blood agar plates and incubated for 18-24 hours at 35-37°C in a 5% CO_2 incubator. Plates with no colonies at 24 hours were incubated for an additional 24 hours before being called negative. Suspected GBS colonies (both hemolytic and non-hemolytic) were tested with catalase reagent and Gram stained. Catalase negative, Gram-positive cocci were then confirmed to be GBS by latex agglutination.

Of the 688 specimens collected, 14 specimens yielded invalid results with the ARIES GBS Assay, of which 13 specimens were resolved upon repeat testing, yielding an invalid rate of 0.15% (1/688). A total of 687 specimens were eligible for analysis. Of the 687 specimens tested, 43 were enriched in Lim broth and then frozen (43/687; 6.3%). The fresh versus frozen comparisons in the clinical study line data and an

analytical study conducted in section **M**.2.*b* above support the inclusion of data derived from frozen specimens in the final results. These results are shown in **Table 8** for all sites combined and for each site separately.

Table 8: Clinical Performance Data for the ARIES GBS Assay vs. Culture for GBS

All Sites			
ARIES GBS	**GBS Culture**		
	Positive	**Negative**	**Total**
Positive	124	48*	172
Negative	5**	510	515
Total	129	558	687

Sensitivity: 96.1% (124/129) 95% CI (91.3%-98.3%)
Specificity: 91.4% (510/558) 95% CI (88.8%-93.5%)

* Of these 48 discordant specimens, 45 were GBS positive when assessed using PCR and bidirectional sequencing (using validated primers) and 3 were GBS negative.

** Of these 5 discordant specimens, 2 were GBS negative when assessed using PCR and bidirectional sequencing and 3 were GBS positive.

Site 1			
ARIES GBS	**GBS Culture**		
	Positive	**Negative**	**Total**
Positive	15	5*	20
Negative	0	90	90
Total	15	95	110
Sensitivity: 100.0% (15/15) 95% CI (79.6%-100.0%) **Specificity:** 94.7% (90/95) 95% CI (88.3%-97.7%)			
* Of these 5 discordant specimens, all 5 were GBS positive when assessed using PCR and bidirectional sequencing.			

Site 2			
ARIES GBS	**GBS Culture**		
	Positive	**Negative**	**Total**
Positive	43	10*	53
Negative	4**	132	136
Total	47	142	189
Sensitivity: 91.5% (43/47) 95% CI (80.1%-96.6%) **Specificity:** 93.0% (132/142) 95% CI (87.5%-96.1%)			
* Of these 10 discordant specimens, 7 were GBS positive when assessed using PCR and bidirectional sequencing and 2 were GBS negative. ** Of these 4 discordant specimens, 2 were GBS negative when assessed using bidirectional sequencing and 2 were GBS positive.			

Site 3			
ARIES GBS	**GBS Culture**		
	Positive	**Negative**	**Total**
Positive	66	33*	99
Negative	1**	288	289
Total	67	321	388
Sensitivity: 98.5% (66/67) 95% CI (92.0%-99.7%) **Specificity:** 89.7% (288/321) 95% CI (85.9%-92.6%)			
* Of these 33 discordant specimens, all 33 were GBS positive when assessed using PCR and bidirectional sequencing. ** This discordant specimen, was confirmed to be GBS positive when assessed using PCR and bidirectional sequencing.			

The external positive and negative quality control isolates used in these studies were diluted in Lim broth and included in the first run performed during each day of

testing. The external positive control was the Zeptometrix *S. agalactiae* Culture Fluid; the external negative control was the Zeptometrix *L. acidophilus* Culture Fluid.

All external GBS positive controls were detected accurately (100.0%, 75/75). All external *L. acidophilus* negative controls were detected accurately (94.9%, 74/78). There were four negative control result failures which were resolved accurately upon repeat testing.

b. Clinical specificity:

See **Table 8** 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 overall prevalence of GBS in patients tested during this study was 18.8% (129/688) based on culture and 25.0% (172/688) based on the ARIES GBS Assay. All clinical specimens collected during this study were collected between May, 2016 and August, 2016.

N. Instrument Name:

ARIES System and 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:

   A barcode reader may be used for entry of sample IDs, or they may be entered manually.

4. Specimen Sampling and Handling:

   Vaginal-rectal Lim broth enriched specimens are manually prepared following the user institution's standard procedures and are transferred to an ARIES GBS Assay cassette for analysis.

5. Calibration:

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

6. Quality Control:

   See section M.1.*c* for information on internal and external controls.
   See section M.3.*a* for information on external control performance during clinical trials.

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.