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

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

I 510(k) Number:

K182467

II Applicant:

DiaSorin Molecular LLC

III Proprietary and Established Names:

Simplexa GBS Direct, Simplexa GBS Positive Control Pack

IV Regulatory Information:

Product Code(s)	Classification	Regulation Section	Panel
NJR	Class I	21 CFR 866.3740 - Streptococcus Spp. Serological Reagents	IM - Immunology & MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

V Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the Simplexa GBS Direct assay and Simplexa GBS Positive Control Pack.

B Measurand:

Conserved region of the *Streptococcus agalactiae* (Group B *Streptococcus*) *cfb* gene.

C Type of Test:

Qualitative real-time DNA amplification and detection assay.

VI Intended Use/Indications for Use:

A Intended Use(s):

Simplexa GBS Direct
The DiaSorin Molecular Simplexa GBS Direct assay is a real-time polymerase chain reaction (PCR) assay intended for use on the LIAISON MDX instrument for the in vitro qualitative detection of Group B Streptococcus (GBS) nucleic acid from 18 to 24 hour Lim broth enrichments of vaginal/rectal specimen swabs obtained from antepartum women. Assay results can be used as an aid in determining the colonization status of antepartum women, but are not intended to diagnose or monitor treatment of a GBS infection.

The Simplexa GBS Direct assay does not provide susceptibility results. Culture isolates are needed to perform susceptibility testing as recommended for penicillin-allergic women.

Simplexa GBS Positive Control Pack
The Simplexa GBS Positive Control Pack is intended to be used as a control with the Simplexa GBS Direct kit. This control is not intended for use with other assays or systems.

B Indication(s) for Use:

Simplexa GBS Direct
The DiaSorin Molecular Simplexa GBS Direct assay is a real-time polymerase chain reaction (PCR) assay intended for use on the LIAISON MDX instrument for the in vitro qualitative detection of Group B Streptococcus (GBS) nucleic acid from 18 to 24 hour Lim broth enrichments of vaginal/rectal specimen swabs obtained from antepartum women. Assay results can be used as an aid in determining the colonization status of antepartum women, but are not intended to diagnose or monitor treatment of a GBS infection.

The Simplexa GBS Direct assay does not provide susceptibility results. Culture isolates are needed to perform susceptibility testing as recommended for penicillin-allergic women.

Simplexa GBS Positive Control Pack
The Simplexa GBS Positive Control Pack is intended to be used as a control with the Simplexa GBS Direct kit. This control is not intended for use with other assays or systems

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For in vitro diagnostic use

D Special Instrument Requirements:

The Simplexa GBS Direct assay is for use with the LIAISON MDX instrument.

VII Device Description:

A Device Description:

The Simplexa GBS Direct assay is an *in vitro* diagnostic, real-time PCR amplification test for the direct, qualitative detection of Group B *Streptococcus* DNA in vaginal/rectal swab specimens from antepartum women that have been incubated for 18-24 hours in Lim enrichment broth. The assay is performed on the LIAISON MDX instrument which is controlled by an external computer equipped with LIAISON MDX Studio Software.

The assay kit comprises frozen PCR mixture containing primers and probes to detect a conserved region of the Group B *Streptococcus* genome and an Internal Control sequence and which is designed to monitor reagent and system integrity.

The Simplexa GBS Direct assay is performed by manually pipetting PCR reagents and test samples into disposables called DADs (Direct Amplification Discs) that comprise eight segments or wedges, each of which may be used to test a separate specimen or control. After sealing the foil on each wedge, the DAD is loaded into the LIAISON MDX instrument and the run is initiated. The system uses a combination of centrifugal force and laser-controlled valves to combine metered aliquots of the unextracted samples and PCR reagents in the assay chamber of each wedge. Amplification, fluorescence-based detection and result interpretation occur automatically.

B Principle of Operation:

The Simplexa GBS Direct assay uses real-time PCR amplification with fluorescently-labeled probes to detect a conserved region of the *S. agalactiae cfb* gene in Lim broth enrichment cultures of vaginal/rectal swabs obtained from pregnant women.

Vaginal/rectal swab specimens are cultured for 18-24 hours in Lim broth after which a sample of the medium is transferred to one wedge of a DAD (Direct Amplification Disc) to which the operator also adds an aliquot of the PCR reagents via a separate port. After sealing the DAD and loading the LIAISON MDX instrument, the sample and PCR mixture are combined for automated amplification, detection and result interpretation. Results are displayed on the computer screen as “GBS Detected”, GBS Not Detected” or “Invalid” (due to failure of the Internal Control or inadequate sample volume) and may be printed.

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☒	☐
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☒	☐
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:	☒	☐

1. Instrument Name:

   LIAISON MDX

2. Specimen Identification:

   Specimens are identified by scanning a bar code label or by typing in an appropriate identifier.

3. Specimen Sampling and Handling:

   The Simplexa GBS Direct assay is for use with Lim broth enrichment cultures of vaginal/rectal swabs that have been incubated for 18-24 hours. To process a sample, an aliquot of the broth culture medium is added directly to the sample port of one wedge/segment of a Direct Amplification Disc (DAD) and an aliquot of PCR mixture is added to the reagent port. The wedge is then sealed and the DAD loaded into the LIAISON MDX instrument.

4. Calibration:

   End-user calibration for the LIAISON MDX instrument is not necessary. Calibration of the optical modules (excitation and emission gain settings) is performed during the manufacturing process and the values are stored in the instrument firmware.

5. Quality Control:

   Refer to Decision Summary **Section X.A(5)** for information on Internal and External Controls.

VIII Substantial Equivalence Information:

A Predicate Device Name(s):
GenePOC GBS LB

B Predicate 510(k) Number(s):

K170557

C Comparison with Predicate:

Device & Predicate Device(s):	K182467	K170557
General Device Characteristic Similarities		
Intended Use/Indications For Use	*Simplexa GBS Direct* The DiaSorin Molecular Simplexa GBS Direct assay is a real-time polymerase chain reaction (PCR) assay intended for use on the LIAISON MDX instrument for the *in vitro* qualitative detection of Group B *Streptococcus* (GBS) nucleic acid from 18 to 24 hour Lim broth enrichments of vaginal/rectal specimen swabs obtained from antepartum women. Assay results can be used as an aid in determining the colonization status of antepartum women, but are not intended to diagnose or monitor treatment of a GBS infection. The Simplexa GBS Direct assay does not provide susceptibility results. Culture isolates are needed to perform susceptibility testing as recommended for penicillin-allergic women. *Simplexa GBS Positive Control Pack* The Simplexa GBS Positive Control Pack is intended to be used as a control with the Simplexa GBS Direct kit. This control is not intended for use with other assays or systems.	The GenePOC GBS LB assay performed on the revogene instrument is a qualitative in vitro diagnostic test designed to detect Group B Streptococcus (GBS) DNA from 18-24 hour LIM broth enrichments of vaginal/rectal specimen swabs obtained from pregnant women. The GenePOC GBS LB assay utilizes automated sample processing and real-time polymerase chain reaction (PCR) to detect a *cfb* gene sequence specific to the *Streptococcus agalactiae* genome. The GenePOC GBS LB assay is indicated for the identification of antepartum GBS colonization and does not provide susceptibility results. It is not intended to diagnose or monitor treatment of GBS infection. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.
Target Analyte	Group B *Streptococcus*	Same
Measurand	Conserved region of the *Streptococcus agalactiae cfb* gene	Same
Sample Type	18-24 hour Lim broth culture of vaginal/rectal swabs from antepartum women	Same
Technology	PCR amplification and real-time fluorescence-based detection performed directly on a sample of Lim broth culture medium	Same

General Device Characteristic Differences		
Instrument System	LIAISON MDX	revogene
PCR Reagent Format	Frozen liquid	Dried

IX Standards/Guidance Documents Referenced:

Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; May 11, 2005.

Guidance for Industry: Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software; January 14, 2005.

Guidance for Industry and FDA Reviewers and Compliance on: Off-the-Shelf Software Use in Medical Devices; September 9, 1999.

X Performance Characteristics (if/when applicable):

A Analytical Performance:

Analytical Studies to characterize the performance of the Simplexa GBS Direct assay were conducted using Lim broth that was inoculated with clinical vaginal/rectal swab matrix, incubated for 18-24 hours and screened as GBS-negative. Samples for testing were prepared by mixing an aliquot of the vaginal/rectal swab culture medium with enumerated stock suspensions of *S. agalactiae*.

1. Precision/Reproducibility:

Reproducibility
The reproducibility of the Simplexa GBS Direct assay between laboratories was evaluated in a study performed by two operators at each of three sites over a period of five days using a panel of contrived samples and Positive/Negative Controls. On each day of testing each operator tested three replicates of each panel member (5 days X 3 sites X 2 operators X 3 replicates = 90 replicates per panel member). The results of the study are summarized in **Table 1**. The Simplexa GBS Direct assay exhibited acceptable reproducibility within and between sites/instruments, days and operators at target levels close to the Limit of Detection (LoD).

Table 1. Summary of results from the Simplexa GBS Direct Reproducibility Study

Sample	Site	Level [1]	Expected Results (%)	Ct Value			
				GBS		Internal Control	
				Mean	%CV	Mean	%CV
Strain ATCC BAA-22	1	Low	30/30 (100)	33.2	1.2		
		Moderate	30/30 (100)	32.6	1.1		
	2	Low	30/30 (100)	33.7	1.7		
		Moderate	30/30 (100)	31.6	1.3		
	4	Low	30/30 (100)	32.1	0.9		
		Moderate	30/30 (100)	31.9	1.1		
	Overall	Low	**90/90 (100)**	**33.0**	**2.4**		
		Moderate	**90/90 (100)**	**32.0**	**1.8**		
Strain ATCC BAA-1138	1	Low	30/30 (100)	34.1	1.6		
		Moderate	30/30 (100)	32.7	1.2		
	2	Low	28/30 (93.3)	34.3 [4]	3.1	31.2 [5]	0.0
		Moderate	30/30 (100)	33.1	1.4		
	4	Low	30/30 (100)	33.1	2.3		
		Moderate	30/30 (100)	31.9	1.5		
	Overall	Low	**88/90 (97.8)**	**33.9**	**2.4**	**31.2**	**0.0**
		Moderate	**90/90 (100)**	**32.6**	**2.0**		
Controls	1	Positive [2]	30/30 (100)	30.2	0.6		
		Negative [3]	30/30 (100)	NA	NA	31.0	0.5
	2	Positive	30/30 (100)	28.7	0.7		
		Negative	30/30 (100)	NA	NA	31.1	0.5
	4	Positive	30/30 (100)	29.1	0.7		
		Negative	30/30 (100)	NA	NA	30.0	0.9
	Overall	Positive	**90/90 (100)**	**29.3**	**2.1**		
		Negative	**90/90 (100)**	**NA**	**NA**	**30.7**	**1.8**

Note: If a positive result for GBS is obtained, detection the Internal Control is not required for a valid result to be reported. Therefore, Ct values for the Internal Control in samples reported positive for GBS were not analyzed (shaded cells)
Ct: Cycle Threshold; %CV: Percent Coefficient of Variation; NA: Not applicable (all samples were negative for GBS and no Ct values were reported)

[1] Low: 1.5X LoD; Moderate: 3.5X LoD (refer to Decision Summary **Section X.A(6)**)
[2] Positive: Simplexa GBS Direct Positive Control
[3] Negative: Uninoculated Lim broth (no vaginal/rectal matrix present)
[4] Excludes Ct values for 2 samples that were reported as GBS negative
[5] Mean of 2 Ct values for samples that were reported as GBS negative

Precision

The precision of the Simplexa GBS Direct assay between different reagent lots was evaluated in a study conducted over a period of three days at a single site using a panel of contrived samples and Positive/Negative Controls. On each day of testing, two replicates of each panel member were tested twice using each of three lots of Simplexa GBS Direct assay reagents (2 replicates X 3 days X 3 lots X 2 runs = 36 replicates per panel member). The results of the study are summarized in **Table 2**. The Simplexa GBS Direct assay exhibited acceptable precision between reagent lots at target levels close to the Limit of Detection (LoD).

Table 2. Summary of results from the Simplexa GBS Direct Precision Study

Sample	Lot	Level	Expected Results (%)	Ct Value			
				GBS		Internal Control	
				Mean	%CV	Mean	%CV
ATCC BAA-22	1	Low	12/12 (100)	32.5	1.6		
		Moderate	12/12 (100)	31.2	1.3		
	2	Low	12/12 (100)	32.2	1.7		
		Moderate	12/12 (100)	31.2	1.4		
	3	Low	12/12 (100)	32.5	2.1		
		Moderate	12/12 (100)	31.2	1.5		
	Overall	**Low**	**36/36 (100)**	**32.4**	**1.8**		
		Moderate	**36/36 (100)**	**31.2**	**1.4**		
ATCC BAA-1138	1	Low	12/12 (100)	33.2	1.0		
		Moderate	12/12 (100)	32.2	2.1		
	2	Low	12/12 (100)	33.1	1.4		
		Moderate	12/12 (100)	31.9	1.3		
	3	Low	12/12 (100)	33.3	2.0		
		Moderate	12/12 (100)	32.1	1.6		
	Overall	**Low**	**36/36 (100)**	**33.2**	**1.5**		
		Moderate	**36/36 (100)**	**32.1**	**1.7**		
Controls	1	Positive	12/12 (100)	28.6	0.9		
		Negative	12/12 (100)	NA	NA	30.0	0.5
	2	Positive	12/12 (100)	28.4	0.6		
		Negative	12/12 (100)	NA	NA	30.6	0.5
	3	Positive	12/12 (100)	28.5	0.6		
		Negative	12/12 (100)	NA	NA	29.4	0.4
	Overall	**Positive**	**36/36 (100)**	**28.5**	**0.7**		
		Negative	**36/36 (100)**	**NA**	**NA**	**30.0**	**1.6**

Note: If a positive result for GBS is obtained, detection the Internal Control is not required for a valid result to be reported. Therefore, Ct values for the Internal Control in samples reported positive for GBS were not analyzed (shaded cells)
Ct: Cycle Threshold; %CV: Percent Coefficient of Variation; NA: Not applicable; all samples were negative for GBS and no Ct values were reported
[1] Low: 2X LoD; Moderate: 4X LoD (refer to Decision Summary **Section X.A(6)**)
[2] Positive: Simplexa GBS Direct Positive Control
[3] Negative: Lim broth enriched vaginal/rectal matrix

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Cross-reactivity
The analytical specificity of the Simplexa GBS Direct assay was evaluated by testing 75 organisms and viruses that were seeded into 18-24 hour GBS-negative Lim broth enrichment cultures of vaginal/rectal swabs (**Table 3**). Unless otherwise noted in the table, the bacteria and yeast species were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL. Parasites and white blood cells were tested at 10^6 cells/mL. A minimum of three assay replicates were evaluated under each test condition. No evidence of cross-reaction with the Simplexa GBS Direct primers and probes was observed.

Table 3. Organisms and viruses evaluated for potential cross-reaction and/or interference with the Simplexa Direct GBS assay

Gram Positive Bacteria (35)	**Gram Negative Bacteria (22)**
Actinomyces israelii	*Acinetobacter baumannii*
Aerococcus viridans	*Aeromonas hydrophila*
Atopobium vaginae	*Bacteroides fragilis*
Bacillus cereus	*Campylobacter jejuni*
Bifidobacterium adolescentis	*Chlamydia trachomatis* [1]
Bifidobacterium breve	*Chromobacterium violaceum*
Brevibacterium linens	*Enterobacter cloacae*
Clostridium difficile	*Escherichia coli*
Clostridium perfringens	*Fusobacterium nucleatum*
Corynebacterium genitalium	*Klebsiella oxytoca*
Enterococcus avium	*Klebsiella pneumoniae*
Enterococcus faecalis	*Mobiluncus curtisii*
Enterococcus faecium	*Mobiluncus mulieris*
Lactobacillus acidophilus	*Neisseria gonorrhoeae*
Lactobacillus crispatus	*Porphyromonas asaccharolytica*
Lactobacillus delbrueckii subsp. *lactis*	*Prevotella melaninogenica*
Lactobacillus iners	*Proteus mirabilis*
Lactobacillus jensensii	*Pseudomonas aeruginosa*
Peptostreptococcus anaerobius	*Salmonella enterica* subsp. *enterica*
Propionibacterium acnes	*Serratia marcescens*
Staphylococcus aureus	*Shigella sonnei*
Staphylococcus epidermidis	*Yersinia enterocolitica*
Staphylococcus saprophyticus	**Mollicutes (3)**
Streptococcus anginosus	*Mycoplasma genitalium* [2]
Streptococcus bovis	*Mycoplasma hominis* [3]
Streptococcus dysgalactiae	*Ureaplasma urealyticum* [3]
Streptococcus intermedius	**Yeasts (5)**
Streptococcus mitis	*Candida albicans*
Streptococcus oralis	*Candida glabrata*
Streptococcus pneumoniae	*Candida parapsilosis*
Streptococcus pyogenes	*Candida tropicalis*
Streptococcus salivarius	*Cryptococcus neoformans*
Streptococcus sanguinis	**Viruses (7)**
Streptococcus suis	Human Immunodeficiency Virus-1 [4]
Streptococcus uberis	Human Papillomavirus (Genotype 18) [5]
Parasites (2)	Herpes Simplex Virus-1
Blastocystis hominis	Herpes Simplex Virus-2
Giardia lamblia	Human Rotavirus [6]
Human DNA (1)	Rhesus Rotavirus
White Blood Cells	Varicella Zoster Virus [7]

[1] 1×10^6 Inclusion Forming Units (IFU)/mL
[2] 5×10^5 Color Change Units (CCU)/mL
[3] 1×10^6 CCU/mL
[4] 5×10^4 Tissue Culture Infectious Dose-50 ($TCID_{50}$)/mL
[5] 1×10^5 International Units (IU)/mL
[6] 1×10^4 $TCID_{50}$/mL
[7] 1×10^5 copies/mL

Because some organisms and viruses were not available for testing or could not be obtained at high concentration, additional *in silico* analysis was performed using the Simplexa GBS Direct primer and probe sequences as queries for organism-specific BLAST (Basic Local Alignment Search Tool) analysis of the NCBI (National Center for Biotechnology

Information) Nucleotide collection (nr/nt) database (**Table 4**). No homology was observed with the targeted organisms and viruses that was considered likely to result in amplification and/or detection.

Table 4. Organisms and viruses evaluated for potential cross-reaction in the Simplexa GBS Direct assay by *in silico* BLAST analysis

Bacteria	**Viruses**
Gardnerella vaginalis	Human Immunodeficiency Virus-1
Mycoplasma genitalium	Human Rotavirus
Parasites	Norovirus Genogroup GI
Trichomonas vaginalis	Norovirus Genogroup GII

Microbial Interference

To evaluate the potential for interference with the Simplexa GBS Direct assay from microbial flora that may be present in vaginal/rectal swab specimens, testing was performed with GBS positive samples in the presence of each of the organisms and viruses that were in the Cross-reactivity Study (**Table 3**), and at the same concentrations. Each species was tested in Lim broth-enriched vaginal/rectal matrix in the presence of two strains of GBS (ATCC BAA-22 and ATCC BAA-1138) at 2X LoD (1.6×10^5 and 6×10^4 CFU/mL, respectively). Testing under each condition was initially performed in triplicate and additional replicates were evaluated in the event of a failure (i.e., a false negative result).

No false negative results were observed in the presence of any of the potentially interfering organisms or viruses with GBS strain ATCC BAA-22. However, with GBS strain ATCC BAA-1138, at least one false negative result was obtained on initial testing with each of the species listed in **Table 5**. The potential for interference by these species with the detection of GBS at low target levels is noted in the Limitations section of the device labeling.

Table 5. Species observed to interfere with detection of low levels of GBS strain ATCC BAA-1138

Interfering Species	**Number Positive/Number Tested**		
	Initial	**Repeat**	**Overall**
Gram Positive Bacteria			
Bacillus cereus	2/3	6/6	8/9 (88.9%)
Clostridium perfringens	2/3	6/6	8/9 (88.9%)
Peptostreptococcus anaerobius	2/3	6/6	8/9 (88.9%)
Staphylococcus saprophyticus	2/3	5/6	7/9 (77.8%)
Streptococcus pyogenes	2/3	6/6	8/9 (88.9%)
Mollicutes			
Ureaplasma urealyticum	1/3	6/6	7/9 (77.8%)
Yeasts			
Candida parapsilosis	1/3	6/6	7/9 (77.8%)

Interfering Substances

The potential for interference with the Simplexa GBS Direct assay was evaluated with a variety of endogenous and exogenous substances that may be present in vaginal/rectal swab specimens (**Table 6**). Each substance was tested in Lim broth enriched vaginal/rectal swab matrix in the presence of two strains of GBS (ATCC BAA-22 and ATCC BAA-1183) at 2X LoD (1.6×10^5 and 6×10^4 CFU/mL, respectively). A minimum of three replicates were tested under each

condition. If interference was observed, additional testing was performed at lower concentrations of the substance to determine at what concentration the adverse effect on assay performance was alleviated.

False negative and/or Invalid results were observed with one or both strains of GBS with fecal fat, meconium, seminal fluid, stool, Gaviscon, Nexium, mesalazine, barium sulfate and the non-steroidal inflammatory drug, Naproxen. Although the potential for interference by these substances is noted in the device labeling, the risk to patients is considered low because of the diluting effect of the Lim broth culture medium on the contents of a vaginal/rectal swab and the high target levels present in GBS positive Lim broth cultures.

Table 6. Substances evaluated for potential interference with the Simplexa GBS Direct assay

Substance	Concentration [1]
Endogenous Biological Materials	
Amniotic Fluid	10% v/v
Fecal fat	5 mg/mL [2]
Meconium	0.31% w/v [2]
Mucus	10% v/v
Seminal fluid	5% v/v [2]
Stool	0.31% w/v [2]
Whole blood	10% v/v
Gastrointestinal Medications	
Antacid: Gaviscon (Aluminum hydroxide/magnesium carbonate)	5% v/v [2]
Antacid: Calcium carbonate	10 mg/mL
Anti-Diarrhea: Imodium (Loperamide hydrochloride)	10% v/v
Anti-Diarrhea: Pepto Bismol (Bismuth salicylate)	10% v/v
Gastritis: Nexium (Esomeprazole magnesium)	0.31 mg/mL [2]
Laxative: Sennosides	5 mg/mL [2]
Anti-inflammatory: Mesalazine	5 mg/mL [2]
Anti-Hemorrhoid Ointment: Hydrocortisone	10 mg/mL
Anti-Hemorrhoid Ointment: Phenylephrine HCl	10 mg/mL
Antimicrobials	
Clotrimazole	10% w/v
Nystatin	10 mg/mL [3]
Topical Agents	
Baby Powder	10 mg/mL
KY Jelly	10% w/v
Mineral oil	10% v/v
Moist Towelettes: Benzalkonium chloride	10% v/v
Moist Towelettes: Ethanol	10% v/v
Other	
Radiology contrast agent: Barium sulfate	5 mg/mL [2]
Non-Steroidal Anti-Inflammatory: Naproxen sodium	3 mg/mL [2]
Spermicidal Lubricant: Nonoxynol-9	10 mg/mL

[1] Highest concentration tested at which all replicates of both strains of GBS produced the expected results
[2] False negative and/or Invalid results were obtained with one or both strains of GBS at higher concentration
[3] On initial testing, 1 sample produced an EC505 error (due to the availability of insufficient information to determine whether amplification occurred); the retest result was acceptable

4. Assay Reportable Range:

Not applicable.

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

Sample Stability
The stability of samples prior to testing with the Simplexa GBS Direct assay was evaluated using 18-24 hour Lim broth cultures of vaginal/rectal swabs that were inoculated with

different concentrations two strains of *S. agalactiae* at levels ranging from 2 to 50X LoD. The samples were held at 2-8°C for different periods prior to testing. At each inoculum concentration, there was a decrease in Ct values over time. The results of the study support the stability of enriched Lim broth cultures of vaginal/rectal swabs for up to 7 days at 2-8°C.

Internal Control
The Simplexa GBS Direct reaction mix includes an Internal Control that is amplified with a different pair of PCR primers than the GBS target and which is detected in a separate optical channel. The Internal Control is designed to monitor for reagent and process integrity.

External Controls

Clinical Study: Positive and Negative External Controls were tested on each LIAISON MDX instrument on each day that patient specimens were evaluated during the prospective Clinical Study (Decision Summary **Section X.C(1)**). The Simplexa GBS Direct Positive Control was used as the Positive External Control. Uninoculated Lim broth was used as the Negative External Control. A total of 68 pairs of Positive and Negative External Controls were tested during the study, all of which (100%) produced the expected results.

Lot-to-Lot Precision: The lot-to-lot precision of the Simplexa GBS Direct Positive Control was evaluated in a study conducted over a period of three days with a single LIAISON MDX instrument and a single lot of Simplexa GBS Direct reaction mixture. On each day of testing, two replicates of each lot of Positive Control were tested twice in different runs (3 days X 2 replicates X 3 lots X 2 runs = 36 replicates). Negative Controls were tested daily to monitor for contamination. All qualitative test results were as expected (**Table 7**) and the Simplexa GBS Direct Positive Control was shown to exhibit acceptable precision between lots.

Table 7. Summary of results from the Simplexa GBS Direct Positive Control Precision Study

Lot	Expected Results (%)	GBS Ct Value	
		Mean	%CV
1	12/12/ (100)	28.9	0.9
2	12/12/ (100)	29.0	0.6
3	12/12/ (100)	29.1	1.3
Overall	36/36 (100)	29.0	1.0

Ct: Cycle Threshold; %CV: Percent Coefficient of Variation

6. Detection Limit:

Limit of Detection
The Limit of Detection (LoD) of the Simplexa GBS Direct assay was established by testing enumerated stocks of two strains of *S. agalactiae* that were diluted in Lim broth-enriched vaginal/rectal swab matrix. For each strain, results from a total of 32 valid assay replicates were obtained at each of eight target concentrations. The study was performed using two lots of Simplexa GBS Direct reagents and four LIAISON MDX instruments. The LoD was defined as the lowest concentration at which ≥95% of replicates produced a positive result. The LoD for ATCC BAA-22 (serotype III) was 80,000 CFU/mL and that for ATCC BAA-1138 (serotype Ia) was 30,000 CFU/mL. These results are acceptable.

Analytical Reactivity/Inclusivity
The inclusivity of the Simplexa GBS Direct assay was evaluated by testing 18 different strains of *S. agalactiae* in addition to those included in the LoD Study (**Table 8**). Each strain was tested in triplicate in Lim broth cultured vaginal/rectal swab matrix at 110,000 CFU/mL (2X the average LoD for strains ATCC BAA-22 and ATCC BAA-1138). All the replicates of each of strain produced positive results. These results are acceptable.

Table 8. Strains of *S. agalactiae* evaluated in the Analytical Reactivity Study

Serotype	ATCC Strain		Serotype	ATCC Strain
Ia	BAA-1177		V	BAA-2672
Ib	51487			BAA-611
Ic	27591		VI	BAA-2671
II	12973		VII	BAA-2670
	BAA-2675		VIII	BAA-2669
III	BAA-1176		IX 1	BAA-2668
	BAA-2674		Non-hemolytic	13813
	12403		Non-hemolytic	BAA-2666
IV	49446			
	BAA-2673			

Bioinformatic Analysis
Bioinformatic analysis using Nucleotide-Nucleotide BLAST (Basic Local Alignment Search Tool) showed that the region of the *cfb* gene targeted by the Simplexa GBS Direct primers and probe is well conserved between strains, with no evidence of mismatches that are predicted to affect the efficiency detection.

7. Assay Cut-Off:

Samples tested with the Simplexa GBS Direct assay are dispositioned as Positive, Negative or Invalid based on the comparison of Cycle Threshold (Ct) values observed in the optical channels for GBS and the Internal Control targets to previously determined cut-offs. The Ct cut-offs were established during development of the assay and validated in the Clinical Study described in Decision Summary **Section X.C(1)**.

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

A study to assess the potential for carry-over/cross-contamination was conducted with the Simplexa Flu A/B & RSV Direct assay (K120413). The study design was determined to be acceptable and no evidence of contamination was observed. Because of the similarities in format and assay workflow with this previously cleared device, no additional contamination studies were conducted with the Simplexa GBS Direct assay.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable.

2. Matrix Comparison:

   Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

   The performance of the Simplexa GBS Direct assay was evaluated in a prospective Clinical Study that was conducted at four (4) sites in the U.S. using de-identified enriched Lim broth cultures of vaginal/rectal swabs from pregnant women that were left over from routine diagnostic testing for GBS. A total of 804 enriched Lim broth cultures were initially enrolled, of which 372 were excluded from the analysis of performance for the following protocol violations: the duration of incubation of the Lim broth was not recorded (n = 239, including all those obtained from Site #3); incubation of the Lim broth culture exceeded 24 hours (n = 115); the sample was a duplicate from a previously enrolled subject (n = 12); testing was performed with the incorrect Simplexa Assay Definition software (n = 4); no Simplexa test result was obtained (n = 1); the subject was male (n = 1). Lim broth cultures of vaginal/rectal swabs from a total of 432 antepartum women at 35-37 weeks of gestation were therefore included in the performance evaluation. The subjects ranged from 18 to 45 years of age.

   Following 18-24 hour incubation of the Lim enrichment broth at each site, separate aliquots were prepared for testing with the Simplexa GBS Direct assay and for shipment to a central laboratory for reference culture and organism identification (i.e., subculture to non-selective blood agar and selective CNA (colistin-nalidixic acid) agar, followed by Gram staining of suspected colonies of *S. agalactiae*, catalase testing (*S. agalactiae* is catalase negative) and latex agglutination using GBS-specific reagents). Agar plates were incubated for a minimum of 48 hours before being discarded as GBS negative. The results of the study are summarized in **Tables 9** and **10**.

   No Invalid results were reported among the 432 samples included in the analysis of performance for the Simplexa GBS Direct assay (Invalid Rate 0% (0/432)).

   These results are acceptable.

Table 9. Performance of the Simplexa GBS Direct assay with Lim broth enrichment cultures of vaginal/rectal swab from antepartum women

		Reference Method		
		Positive	**Negative**	**Total**
Simplexa GBS Direct	**Positive**	97	13 [1]	**110**
	Negative	3 [2]	319	**322**
	Total	**100**	**332**	**432**
Sensitivity		97.0% (97/100); 95% CI: 91.5-99.0%		
Specificity		96.1% (319/332); 95% CI: 93.4-97.7%		
Positive Predictive Value		88.2% (97/110)		
Negative Predictive value		99.1% (319/322)		

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

[1] 11/13 (84.6%) samples were reported positive for GBS by an alternative FDA-cleared molecular method

[2] 3/3 (100%) samples were reported negative for GBS by an alternative FDA-cleared molecular method

Table 10. Performance of the Simplexa GBS Direct assay with Lim broth enrichment cultures of vaginal/rectal swab from antepartum women, stratified by study site

Site #	**Culture Positive (%)**	**Simplexa GBS Direct (%; 95% CI)**	
		Sensitivity	**Specificity**
1	47/168 (28.0)	45/47 (95.7; 85.8-98.8)	117/121 (96.7; 91.8-98.7)
5	37/208 (17.8)	36/37 (97.3; 86.2-99.5)	163/171 (95.3; 91.0-97.6)
6	16/56 (28.6)	16/16 (100; 80.6-100)	39/40 (97.5; 87.1-99.6)
Overall	**100/432 (23.1)**	**97/100 (97.0; 91.5-99.0)**	**319/332 (96.1; 93.4-97.7)**

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

Note: All Lim broth cultures from study Site #3 were excluded from the analysis of performance because the duration of incubation prior to testing with the Simplexa GBS Direct assay was not recorded as stipulated in the study protocol

2. Clinical Specificity:

Refer to Decision Summary **Section X.C(1)**, 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 Simplexa GBS Direct assay was evaluated in a prospective Clinical Study of pregnant women at 35-37 weeks of gestation with samples from three (3) sites in the

U.S. (Decision Summary **Section X.C(1)**). The overall prevalence of GBS colonization as determined by the Simplexa GBS Direct assay was 25.5% (110/432), whereas by conventional culture it was 23.1% (100/432). In **Table 11**, the prevalence of GBS colonization as determined by the Simplexa GBS Direct assay is shown stratified by clinical site.

Table 11. Observed prevalence of GBS colonization in pregnant women as determined by the Simplexa GBS Direct assay, stratified by clinical site

Site #	Number of Samples	Simplexa GBS Direct Positive	Percent Prevalence
1	168	49	29.2
5	208	44	21.2
6	56	17	30.4
Overall	**432**	**110**	**25.5**

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

XI Proposed Labeling:

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

XII Conclusion:

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