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

A. 510(k) Number:

k121539

B. Purpose for Submission:

To obtain substantial equivalence determination for the Xpert® GBS LB Assay

C. Measurand:

3´ untranslated region of the *cfb* gene of *S. agalactiae* (Group B *Streptococcus*, GBS)

D. Type of Test:

Real Time Polymerase Chain Reaction (PCR)

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert® GBS LB Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3740 – Streptococcal spp. serological reagents

   21 CFR 862.2570 – Instrumentation for clinical multiplex test systems

2. Classification:

   Class I (Not exempt), Class II

3. Product code:

   NJR - Nucleic Acid Amplification Assay System, Group B Streptococcus, Direct Specimen

OOI – Real-time Nucleic Acid Amplification

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The Cepheid Xpert GBS LB Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test designed to detect Group B *Streptococcus* (GBS) DNA from enriched vaginal/rectal swab specimens, using fully automated real-time 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.

2. Indication(s) for use:

   The Cepheid Xpert GBS LB Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test designed to detect Group B *Streptococcus* (GBS) DNA from enriched vaginal/rectal swab specimens, using fully automated real-time 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.

3. Special conditions for use statement(s):

   - Prescription Use Only

- Careful compliance with the instructions in the package insert and to the Vaginal/Rectal Specimen Collection Protocol instructions document is necessary to avoid erroneous results.

4. Special instrument requirements:

   GeneXpert Instrument Systems

   - GeneXpert Dx
   - GeneXpert Infinity-48
   - GeneXpert Infinity-80

I. Device Description:

The Cepheid Xpert GBS LB Assay is an automated *in vitro* diagnostic DNA test for the qualitative detection of Group B Streptococcus (GBS, *S. agalactiae*) DNA from enriched vaginal/rectal swab specimens, using fully automated real-time polymerase chain reaction (PCR) with fluorogenic detection of the amplified DNA. The assay is performed on the Cepheid GeneXpert® Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample lysis, nucleic acid purification and amplification, and detection of the target sequence in complex samples using real-time PCR (Polymerase chain reaction) assays. The GeneXpert Instrument System family comprises a GeneXpert Dx instrument (GX-I, GX-IV, GX-XVI), the GeneXpert Dx XVI available with 4, 8, 12, or 16 modules; a GeneXpert Infinity-48, available with 16, 24, 32, 40 or 48 modules, or a GeneXpert Infinity-80 available with 16, 24, 32, 40, 48, 56, 64, 72, or 80 modules. The individual testing modules are identical for all instrument systems. The instrument systems also contain a computer, and preloaded software for running tests and viewing the results. The GeneXpert Infinity Systems contain robotic features for cartridge handling.

The Xpert GBS LB Assay includes reagents pre-loaded in the Xpert GBS LB cartridge for the simultaneous detection of the target GBS DNA. A Sample Processing Control (SPC), an Internal Control (IC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target DNA; the IC is present to monitor the presence of inhibitors in the PCR reaction. The PCC verifies reagent rehydration, PCR-tube filling in the cartridge, probe integrity, and dye stability. The GBS primers and probe detect a target within a 3´ DNA region adjacent to the *cfb* gene of *S. agalactiae*.

After collecting and transporting a swab sample to the laboratory, the swab is placed in Lim broth for enrichment overnight, after which a clean swab (Cepheid part number SDPS-120) dipped into the enrichment broth specimen is transferred to the designated chamber of the cartridge. The GeneXpert Instrument System performs sample preparation by eluting the specimen material from the swab, mixing the sample with the SPC (*Bacillus globigii* in the form of a bead within the cartridge) and treatment reagent, capturing cellular material on a

filter, lysing the cells, and eluting the DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the integrated reaction tube for real-time PCR and detection. The results are interpolated by the GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms. Results may be viewed and printed. The test process takes approximately 55 minutes. Sample preparation, amplification, and real-time detection are all fully automated and completely integrated.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Cepheid Smart GBS

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

   K062948

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Identification of GBS in enriched Lim Broth specimens from antepartum women	Identification of GBS in enriched Lim Broth specimens and direct vaginal/rectal swabs from antepartum and intrapartum women
Organism Detection	Group B Streptococcus	Same
Collection and Transport Media	Cepheid Collection Device or swab in a non- nutritive transport medium	Same
Assay Format	Amplification: PCR with ICORE heating and cooling module. Detection: Fluorogenic target specific hybridization	Same
DNA Target Sequence	3´ untranslated region of the *cfb* gene	Same
Self-Contained System Assay	Yes	Yes, after sample preparation which is off-line.
Single Use	Yes; single-use Cepheid cartridge includes integrated reaction tube	Yes; single-use reaction tubes
Automated	Yes	Same

Similarities		
Item	Device	Predicate
amplification/detection and result interpretation		
Time to Result	≤ 55 minutes total after sample addition to cartridge	~ 75 minutes total including sample preparation and addition to reaction tube
Criteria for Ct Determination	Primary Growth Curve	Same

Differences		
Item	Device	Predicate
Specimen Type	From 18-24 hour Lim Broth cultures of vaginal/rectal swabs	Direct from vaginal/rectal swab or from 18-24 Lim broth cultures of vaginal/rectal swabs
Assay Platform	Cepheid GeneXpert Dx System, GeneXpert Infinity-48 System, GeneXpert Infinity-80 System	Cepheid SmartCycler System
Sample Preparation	Automated Sample Preparation after swab specimen is placed in Lim broth for 18-24 hours at $35^o – 37^o$ C.	Manual sample preparation With enriched option, swab specimen is placed in Lim broth overnight at 37^o C.
External Assay Controls	Materials available, but not required.	Materials available and required.
Built in Lysis Control	Yes	N/A, the negative and positive controls do not go through sample preparation steps.
Sample Processing Control	Sample Processing Control; Failures result in single sample repeat.	Uses external controls for sample Processing Control
Internal Assay Controls	Sample Processing Control; Internal control; Probe Check (all optical channels) Failures result in single sample repeat.	Internal Control
Fluidics	Self-contained	Manual Sample Preparation

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

- CLSI EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*; Approved Guideline –Second Edition
- *Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable*, FDA, April 25, 2006.
- EN 13640, *Stability Testing of in vitro Diagnostic Reagents*, June 2002
- ASTM D4169-05, *Standard Practice for Performance Testing of Shipping Containers and Systems*
- *Draft Guidance for Industry and Food and Drug Administration Staff, 510(k) Device Modification: Deciding When to Submit a 510(k) for a Change to an Existing Device*, Draft; July 27, 2011.
- *Guidance for Industry and FDA Staff – Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems*, issued on March 10, 2005.
- *Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices* – Guidance for Industry and FDA Staff, issued on May 11, 2005.
- *Guidance for Industry – Cybersecurity for Networked Medical Devices Containing Off-the Shelf (OTS) Software*, issued January 14, 2005.
- *General Principles of Software Validation; Final Guidance for Industry and FDA Staff,* issued January 11, 2002.

L. Test Principle:

The GeneXpert instrument systems automate and integrate sample lysis, nucleic acid purification and amplification, and detection of the target sequence in complex samples using real-time and reverse transcription Polymerase Chain Reaction (RT-PCR) and PCR assays. The systems consist of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination concerns are minimized.

The Xpert GBS LB Assay includes reagents for the simultaneous detection of the target GBS DNA, a sample-processing control (SPC) to monitor processing conditions, and an internal control (IC) to monitor PCR conditions and the absence of reaction inhibition. The probe check feature verifies reagent rehydration, PCR-tube filling in the cartridge, probe integrity, and dye stability. The GBS primers and probe detect a target within a 3′ DNA region adjacent to the *cfb* gene of *S. agalactiae*.

After collecting and transporting a swab sample to the laboratory, the swab is placed in Lim broth for enrichment overnight at 35° – 37°C. A clean swab is dipped into the Lim broth after enrichment and is then transferred to the designated chamber of the cartridge. The GeneXpert System performs sample preparation by eluting the specimen material from the swab, mixing the sample reagent with the SPC (*Bacillus globigii* in the form of a bead within the cartridge) and treatment reagent, capturing cellular material on a filter, lysing the cells, and eluting the

DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the integrated reaction tube for real-time PCR and detection. The results are interpolated by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms. Results may be viewed and may be printed. The test process takes approximately 55 minutes or less.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility Study: A panel of seven samples with varying concentrations of two different GBS strains (ATCC # 13813 GBS Type II and ATCC # 49446 GBS Type IV) were tested by 2 operators each in triplicate on 5 different days at 3 sites for a total of 90 replicates tested for each sample (7 panel members x 2 operators x 3 times/day x 5 days x 3 sites). One lot of Xpert GBS LB was used at each of the 3 testing sites. Testing of the Xpert GBS LB Assays was performed on the GeneXpert Infinity-48 at Site 1, GeneXpert Dx at Site 2 and GeneXpert Infinity-80 at Site 3.

Known concentrations of GBS cell stocks were serially diluted in a simulated background matrix to achieve moderate positive (~3-4 X LOD) and low positive (~1X LOD) concentrations. The high negative samples were prepared by further diluting "low positive" samples to a concentration below the LoD such that results of repeated tests would be negative approximately 20% to 80% of the time. All study participants were blinded to the identity and expected result for each sample.

External negative and GBS positive controls were run on each day that samples were tested. Study samples were not run until correct results were obtained for both the negative and positive controls.

Xpert GBS LB testing yielded initial valid results 95.6% of the time with 10 ERROR and 18 NO RESULT outcomes for which repeat testing was performed. Twenty seven of 28 repeated tests yielded valid results with an overall rate of assay success in this study of 99.8% (629/630.)

All of the negative (89/89) study samples were correctly classified as negative. All of the moderate positives for both GBS strains 1 and Strain 2 (90/90) study samples were correctly classified as positive. The low positive samples were prepared at concentrations near the LoD to target a positive result ~95% of the time. The GBS strain 1 low positive samples yielded a positive result in 98.9% (89/90) of the runs. The GBS strain 2 low positive samples yielded a positive result in 100.0% (90/90) of the runs.

The high negative samples were prepared to target a negative result ~ 20% to 80% of the time. The GBS strain 1 high negative samples yielded a negative result in 72.2% (65/90) of the runs. The GBS strain 2 high negative sample yielded a negative result in 80.0% (72/90) of the runs. The results are summarized by instrument in the following table.

Summary of Reproducibility Results

Specimen ID	Site 1 (Infinity-48)	Site 2 (GeneXpert Dx)	Site 3 (Infinity-80)	% Total Agreement by Sample
GBS strain 1 moderate positive	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
GBS strain 1 low positive	100.0% (30/30)	96.7% (29/30)	100.0% (30/30)	98.9% (89/90)
GBS strain 1 high negative	73.3% (22/30)	80.0% (24/30)	63.3% (19/30)	72.2% (65/90)
GBS strain 2 moderate positive	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
GBS strain 2 low positive	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
GBS strain2 high negative	80.0% (24/30)	83.3% (25/30)	76.7% (23/30)	80.0% (72/90)
negative	100.0% (30/30)	100.0% (29/29)[a]	100.0% (30/30)	100.0% (89/89)[a]

[a]One negative sample had indeterminate result on initial test but was not retested by mistake.

A numerical comparison of Ct value results by target in each sample level between the GeneXpert, Infinity 48 and Infinity 80 and their overall results are provided in the following table. Study results demonstrated that there were no significant differences in assay performance between the three instruments.

Summary of Ct Value Results by Instrument and Sample Concentration

GBS strain 1 - moderate positive				
		SPC	GBS	IC
Infinity-48	MEAN	33.04	35.85	30.39
n = 30	STDEV	0.50	0.47	0.19
	CV	1.52%	1.30%	0.64%
No. of results		29	30	30

GeneXpert Dx	MEAN	32.98	35.82	30.51
n = 30	STDEV	0.62	0.64	0.25
	CV	1.90%	1.79%	0.83%
No. of results		30	30	30
Infinity-80	MEAN	32.95	36.05	30.48
n = 30	STDEV	0.61	0.96	0.22
	CV	1.85%	2.67%	0.71%
No. of results		30	30	30
Overall	MEAN	32.99	35.91	30.46
n = 90	STDEV	0.58	0.72	0.23
	CV	1.75%	2.00%	0.74%
No. of results		89	90	90
GBS strain 1 - low positive				
		SPC	GBS	IC
Infinity-48	MEAN	32.65	37.13	30.41
n = 30	STDEV	0.50	0.78	0.23
	CV	1.54%	2.10%	0.75%
No. of results		30	30	30
GeneXpert Dx	MEAN	32.96	37.50	30.46
n = 30	STDEV	0.61	1.25	0.33
	CV	1.84%	3.33%	1.07%
No. of results		30	29	30
Infinity-80	MEAN	32.80	37.41	30.54
n = 30	STDEV	0.50	0.73	0.36
	CV	1.54%	1.94%	1.19%
No. of results		30	30	30
Overall	MEAN	32.80	37.35	30.47
n = 90	STDEV	0.55	0.95	0.31
	CV	1.67%	2.54%	1.03%
No. of results		90	89	90

GBS strain 1 – high negative				
		SPC	GBS	IC
Infinity-48	MEAN	32.77	N/A	30.43
n = 30	STDEV	0.50	N/A	0.22
	CV	1.54%	N/A	0.71%
No. of results		30		30
GeneXpert Dx	MEAN	32.82	N/A	30.47
n = 30	STDEV	0.43	N/A	0.20
	CV	1.31%	N/A	0.66%
No. of results		30		30
Infinity-80	MEAN	32.80	N/A	30.45
n = 30	STDEV	0.49	N/A	0.24
	CV	1.51%	N/A	0.80%
No. of results		30		30
Overall	MEAN	32.79	N/A	30.45
n = 90	STDEV	0.47	N/A	0.22
	CV	1.44%	N/A	0.72%
No. of results		90		90
GBS strain 2 - moderate positive				
		SPC	GBS	IC
Infinity-48	MEAN	32.93	35.6	30.46
n = 30	STDEV	0.40	0.57	0.18
	CV	1.22%	1.60%	0.58%
No. of results		30	30	30
GeneXpert Dx	MEAN	32.98	35.63	30.38
n = 30	STDEV	0.51	0.63	0.23
	CV	1.56%	1.76%	0.75%
No. of results		30	30	30
Infinity-80	MEAN	32.89	35.50	30.43
n = 30	STDEV	0.74	0.59	0.28
	CV	2.24%	1.66%	0.92%
No. of results		30	30	30
Overall	MEAN	32.93	35.58	30.42
n = 90	STDEV	0.56	0.59	0.23
	CV	1.71%	1.66%	0.76%
No. of results		90	90	90

GBS strain 2 - low positive				
		SPC	GBS	IC
Infinity-48	MEAN	32.90	37.05	30.39
n = 30	STDEV	0.64	1.19	0.24
	CV	1.95%	3.20%	0.79%
No. of results		30	30	30
GeneXpert Dx	MEAN	33.01	36.97	30.46
n = 30	STDEV	0.66	0.75	0.29
	CV	1.99%	2.04%	0.94%
No. of results		30	30	30
Infinity-80	MEAN	32.60	36.96	30.42
n = 30	STDEV	0.52	0.84	0.28
	CV	1.59%	2.26%	0.91%
No. of results		30	30	30
Overall	MEAN	32.84	36.99	30.42
n = 90	STDEV	0.63	0.93	0.27
	CV	1.91%	2.53%	0.88%
No. of results		90	90	90
GBS strain 2 – high negative				
		SPC	GBS	IC
Infinity-48	MEAN	32.79	N/A	30.49
n = 30	STDEV	0.49	N/A	0.25
	CV	1.51%	N/A	0.82%
No. of results		30		30
GeneXpert Dx	MEAN	32.90	N/A	30.39
n = 30	STDEV	0.40	N/A	0.26
	CV	1.23%	N/A	0.86%
No. of results		30		30
Infinity-80	MEAN	33.00	N/A	30.48
n = 30	STDEV	0.60	N/A	0.24
	CV	1.81%	N/A	0.80%
No. of results		30		30
Overall	MEAN	32.90	N/A	30.45
n = 90	STDEV	0.51	N/A	0.25
	CV	1.54%	N/A	0.83%
No. of results		90		90

Negative				
		SPC	GBS	IC
Infinity-48	MEAN	32.87	N/A	30.45
n = 30	STDEV	0.51	N/A	0.21
	CV	1.55%	N/A	0.68%
No. of results		30		30
GeneXpert Dx	MEAN	32.85	N/A	30.39
n = 29	STDEV	0.54	N/A	0.32
	CV	1.65%	N/A	1.04%
No. of results		29		29
Infinity-80	MEAN	32.83	N/A	30.47
n = 30	STDEV	0.68	N/A	0.27
	CV	2.06%	N/A	0.87%
No. of results		30		30
Overall	MEAN	32.85	N/A	30.44
n = 89	STDEV	0.58	N/A	0.27
	CV	1.75%	N/A	0.87%
No. of results		89		89

Precision Study:

An in-house precision study was conducted to compare the performance of the GeneXpert D and the Infinity-80 instrument systems. A panel of 7 samples with varying concentrations of 2 different GBS strains (ATCC # 13813 GBS Type II and ATCC # 49446 GBS Type IV) was tested on 12 different days by 2 operators. The 7 panel members used for this study contained the same organisms and concentration levels and were prepared in the same manner as those used in the reproducibility study. Each operator conducted 4 runs of each panel specimen per day on each of the 2 instrument systems (7 specimens x 4 times/ day x 12 days x 2 operators x 2 instrument systems). One lot of Xpert GBS LB Assay was used for the study. All study participants were blinded to the identity and expected result for each sample.

For the panel specimens, the cartridges were prepared by adding the sample according to instructions in the package insert instructions. The prepared cartridges were then subjected to an upfront hold time of 60 minutes (maximum hold time allowed by the system) before analyzing on each platform. For the GeneXpert, cartridges were loaded into the appropriate module and the test was initiated 60 minutes after cartridge preparation. For the Infinity, the prepared cartridges were

scanned on the barcode reader, placed onto the accumulator belt, and the test initiated 60 minutes after preparation. In addition to challenging both instruments with the 60 minute delay in test initiation, this precision study was designed to challenge the "worst case" situation for the Infinity-80 System which entailed loading of test cartridges continuously (28 plus 28) by the 2 operators onto the Infinity instrument conveyor belt.

External negative and GBS positive controls were run by each operator on each day that samples were tested. Study samples were not run until correct results were obtained for both the negative and positive controls.

Xpert GBS LB assays for 99.1% (1332/1344) of samples were successful on the first attempt. All 12 samples that gave error or invalid results yielded expected results upon repeat testing. All negative samples yielded negative results and all moderate positive samples yielded positive results as expected. The low positive samples which were prepared at a ~1X LoD concentration level, yielded positive results for strain #1 100% of the time and for strain #2, 98.4% of the time. The high negative samples were prepared at a concentration level that would produce negative results approximately 20-80% of the time. GBS strain #1 produced a negative result in 76.6% of tests and strain 2, 83.9% of tests.

Results from this study demonstrated that there were no significant differences in assay performance between the 2 instrument systems for any of the panel concentration levels. The study results validated the potential worst case sample hold times (maximum wait time of one hour) on the Infinity System. Results from this study are summarized by instrument in the following table.

Summary of Instrument Precision Results

Specimen ID	GeneXpert Dx	Infinity-80	% Total Agreement by Sample
GBS strain 1 moderate positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
GBS strain 1 low positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
GBS strain 1 high negative	77.1% (74/96)	76.0% (73/96)	76.6% (147/192)
GBS strain 2 moderate positive	100.0% (96/96)	100.0% (96/96)	100.0% (192/192)
GBS strain 2 low positive	99.0% (95/96)	97.9% (94/96)	98.4% (189/192)
GBS strain2 high negative	85.4% (82/96)	82.3% (79/96)	83.9% (161/192)
	100.0%	100.0%	100.0%

negative	(96/96)	(96/96)	(192/192)

A comparison of Ct value results by target in each sample level between the GeneXpert Dx system and Infinity- 80 system and their overall results are provided in the following table.

Summary of Ct Value Results by Target in Each Sample Level

GBS strain 1 - moderate positive				
		SPC	GBS	IC
Infinity	MEAN	32.83	35.93	30.45
n = 96	STDEV	0.52	0.75	0.41
	CV	1.59%	2.08%	1.35%
No. of results		96	96	96
GeneXpert Dx	MEAN	32.72	35.88	30.48
n = 96	STDEV	0.63	0.77	0.29
	CV	1.93%	2.14%	0.94%
No. of results		96	96	96
Overall	MEAN	32.77	35.90	30.47
n = 192	STDEV	0.58	0.75	0.35
	CV	1.77%	2.10%	1.16%
No. of results		192	192	192
GBS strain 1 - low				
		SPC	GBS	IC
Infinity	MEAN	32.73	37.67	30.40
n = 96	STDEV	0.49	1.08	0.28
	CV	1.51%	2.86%	0.92%
No. of results		96	96	96
GeneXpert Dx	MEAN	32.73	37.64	30.42
n = 96	STDEV	0.39	1.04	0.22
	CV	1.19%	2.76%	0.71%
No. of results		96	96	96
Overall	MEAN	32.73	37.65	30.41
n = 192	STDEV	0.44	1.06	0.25
	CV	1.35%	2.81%	0.82%
No. of results		192	192	192
GBS strain 1 – high negative				
		SPC	GBS	IC

Infinity	MEAN	32.75	N/A	30.39
n = 96	STDEV	0.64	N/A	0.25
	CV	1.95%	N/A	0.84%
No. of results		96		96
GeneXpert Dx	MEAN	32.61	N/A	30.42
n = 96	STDEV	0.54	N/A	0.29
	CV	1.64%	N/A	0.95%
No. of results		96		96
Overall	MEAN	32.68	N/A	30.40
n = 192	STDEV	0.59	N/A	0.27
	CV	1.81%	N/A	0.89%
No. of results		192		192
GBS strain 2 - moderate positive				
		SPC	GBS	IC
Infinity	MEAN	32.62	35.6	30.39
n = 96	STDEV	0.99	0.51	0.23
	CV	3.05%	1.43%	0.76%
No. of results		96	96	96
GeneXpert Dx	MEAN	32.82	35.63	30.44
n = 96	STDEV	0.49	0.52	0.29
	CV	1.51%	1.47%	0.95%
No. of results		96	96	96
Overall	MEAN	32.72	35.61	30.42
n = 192	STDEV	0.79	0.51	0.26
	CV	2.41%	1.44%	0.86%
No. of results		192	192	192
GBS strain 2 - low				
		SPC	GBS	IC
Infinity	MEAN	32.8	37.06	30.48
n = 96	STDEV	0.68	1.00	0.69
	CV	2.07%	2.69%	2.25%
No. of results		96	94	96
GeneXpert Dx	MEAN	32.65	36.92	30.44
n = 96	STDEV	0.56	0.94	0.28
	CV	1.72%	2.56%	0.92%
No. of results		96	95	96
Overall	MEAN	32.72	36.99	30.46
n = 192	STDEV	0.63	0.97	0.52
	CV	1.91%	2.63%	1.72%
No. of results		192	189	192
GBS strain 2 – high negative				
		SPC	GBS	IC

Infinity	MEAN	32.7	N/A	30.39
n = 96	STDEV	0.54	N/A	0.22
	CV	1.67%	N/A	0.73%
No. of results		96		96
GeneXpert Dx	MEAN	32.79	N/A	30.46
n = 96	STDEV	0.58	N/A	0.26
	CV	1.78%	N/A	0.85%
No. of results		96		96
Overall	MEAN	32.74	N/A	30.39
n = 192	STDEV	0.56	N/A	0.22
	CV	1.72%	N/A	0.80%
No. of results		192		192
Negative				
		SPC	GBS	IC
Infinity-80	MEAN	32.67	N/A	30.39
n = 96	STDEV	0.54	N/A	0.21
	CV	1.64%	N/A	0.67%
No. of results		96		96
GeneXpert Dx	MEAN	32.68	N/A	30.39
n = 96	STDEV	0.50	N/A	0.30
	CV	1.53%	N/A	0.97%
No. of results		96		96
Overall	MEAN	32.68	N/A	30.39
n = 192	STDEV	0.52	N/A	0.25
	CV	1.58%	N/A	0.84%
No. of results		192		192

b. Linearity/assay reportable range:

Not Applicable, The Xpert GBS LB Assay is a qualitative assay.

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

Specimen Stability

Vaginal/rectal swab stability: The stability of vaginal/rectal swab specimens was evaluated at room temperature for up to 24 hours and up to 7 days at 2-8ºC. Positive and negative swab samples were prepared using a background matrix. Two serotypes of GBS were evaluated with positive samples containing 5x the LoD of the Xpert GBS LB assay. Swabs were stored at 2ºC, 8ºC and 25ºC for the claimed storage times, followed by inoculation and incubation of Lim broths. A total of 325 tests were performed on 15 Xpert instruments. Under the conditions of this study, all positive and negative swab samples were identified correctly, and there was no significant difference in performance at any time point tested.

Lim Broth Stability: The stability of enriched Lim broths was evaluated at T=0 and daily after storage of up to 4 days after storage at 2ºC, 8ºC, and 25ºC followed by testing with the Xpert GBS LB assay. Two serotypes of GBS were evaluated with preparation of positive samples using swabs spiked with background matrix and GBS organisms at 5x the LoD, followed by placement into Lim broth media. Negative swab samples were prepared with matrix and placed into Lim broth for incubation. Lim broth samples were incubated for 18 hours at 35ºC and then aliquotted and stored at 2ºC, 8ºC, and 25ºC for the specified storage times. A total of 205 tests were performed in this study resulting in all positive (both Serotypes II and IV) and negative specimens correctly identified using the Xpert GBS LB Assay for up to 4 days of storage at 2-8ºC. No significant difference in results was observed between serotypes, storage temperatures, or storage times.

For the most extreme storage conditions (swab storage at 2-8ºC for 7 days followed by storage of the enriched Lim broth at 2ºC for 4 days before testing with the Xpert GBS LB assay), a total of 36 samples were tested with all tests yielding correct results and no significant difference in results.

Cartridge Hold Time Stability for Infinity Instruments: An analytical study was performed to evaluate the maximum cartridge hold time of one hour for the GBS LB Assay product between sample addition and cartridge processing. Positive samples were prepared using GBS diluted in Lim broth with background matrix to simulate clinical samples. Negative samples consisted of background matrix in Lim broth. A total of 288 tests were performed at T=0 and T= 1 hour and all completed positive and negative specimens were correctly identified using the Xpert GBS LB Assay. There was no significant difference in results between testing at T= 0 versus T=1 hour. The data from this study supports a 1 hour maximum hold time for inoculated Xpert GBS LB cartridges prior to testing on the Infinity Instruments.

Controls

Sample Processing Control (SPC): The SPC verifies that specimen processing is adequate for each cartridge tested. The SPC which is included in each cartridge consists of the organism *Bacillus globigii* in the form of a dry bead. The SPC PASSES if a valid cycle threshold (Ct) is generated in a negative sample. The SPC may not amplify in a high-positive specimen. If the SPC fails, the test result will be INVALID and the test should be repeated.

Internal Control (IC): The IC is present in each reaction to verify functional PCR reagents and the absence of inhibition that may prevent PCR amplification. In any test, where the GBS result is negative, the growth curve for the IC must meet specific criteria of cycle threshold (Ct) value and Endpoint Fluorescence (EPF) value to be considered acceptable. If the IC fails, the test result will be INVALID and the test should be repeated.

Probe Check Control (PCC): Before the start of the PCR reaction, the GeneXpert

Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Fluorescence readings are obtained at multiple temperatures. These readings are then compared to default settings established by Cepheid. The PCC is considered to PASS if the fluorescence generated meets the validated acceptance criteria. If the PCC fails for GBS target, IC or SPC, a probe check error is reported and the specimen should be retested.

External Controls: One external positive control (NATtrol positive, *S. agalactiae*) and one external negative control (NATtrol negative, *L. acidophilus*) were run on each day of the analytical and clinical studies. Testing with the Xpert GBS LB assay was not performed until these external controls provided the expected correct results.

d. Detection limit:

A study was performed to determine the limit of detection (LoD) of the Xpert GBS LB Assay with 11 GBS strains representing 9 known GBS serotypes. The LoD is defined as the number of colony forming units (CFU/ml) of GBS that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which a minimum of 19 of 20 replicates were positive.

The strains tested were diluted into a simulated sample matrix. The LoD was determined by testing a total of 20 replicates at 3-4 concentrations per strain as well as testing 20 negative samples. All concentrations were verified by plating on Tryptic Soy Agar plates incubated at 37ºC for 18-24 hours.

Testing of all 11 GBS isolates yielded positive results as expected, confirming analytical inclusivity. The estimated LoD and confidence intervals were determined using logistic regression over a range of 3-4 concentrations (CFU/mL.)

The LoD was determined to be 333 CFU/mL for 8 of 11 strains tested. The LoD for 3 additional strains was determined to be 173 CFU/mL, 76 CFU/mL, and 133 CFU/mL. The confirmed LoD and LoD point estimates with 95% upper and lower confidence intervals for each strain tested are provided in the following table.

Confirmed LoD – GBS Serotypes

Strain ID	Confirmed LoD (CFU/swab) [at least 19/20 positive]	Confirmed LoD (CFU/mL of Lim Broth) [at least 19/20 positive]	LoD Estimate (Logistic Regression) (CFU/swab)		
			Lower 95% CI	LoD Estimate	Upper 95% CI
Serotype Ia	13 (20/20)	173	8.0	10.0	14.2
Serotype Ib	25 (20/20)	333	8.7	11.1	15.7
Serotype II	25 (20/20)	333	10.4	13.3	20.1
Serotype II	25 (20/20)	333	20.1	23.6	32.1
Serotype III	25 (19/20)	333	16.3	21	35.4
Serotype IV	25 (20/20)	333	10.7	14.4	23.7
Serotype IVc	5 (20/20)	67	2.4	3.1	4.8
Serotype V	25 (20/20)	333	14.2	18.2	26.1
Serotype VI	25 (20/20)	333	7.6	10.4	17.8
Serotype VII	25 (20/20)	333	10.2	13.4	20.7
Serotype VIII	10 (20/20)	133	4.3	5.6	8.4

e. Inclusivity

See LoD studies above.

f. Interference

The potentially interfering endogenous and exogenous substances listed in the table below were tested in an analytical study. Substances were tested at concentrations close to saturation.

Negative samples (n = 8) were tested per substance to determine the effect on the performance of the sample processing control (SPC) and internal control (IC). Positive samples (n=8) were tested per substance with 2 GBS serotypes (II and IV) at 2 levels: 3X (75 CFU/swab) and 5X (125 CFU/swab) the LoD. Statistical significance was determined by comparing cycle threshold (Ct) values from tests run in the presence and in the absence of each substance.

Results of the study yielded no significant inhibitory effects for both GBS negative and GBS positive specimens in the presence of any of the interfering substances. All replicates of positive specimens using GBS serotype II and IV were correctly reported as GBS POSITIVE at GBS levels 3X and 5X LoD using the Xpert GBS LB Assay. All GBS-negative specimens yielded the correct negative result.

The lubricating gel had an inhibitory effect on the performance of the SPC in negative samples (average Ct of 35.9 for sample with lubricating gel versus 32.6 for the Lim Broth Control); however, correct assay results for both positive and negative samples were obtained.

Potentially Interfering Substances in Xpert GBS LB Assay

Category	Substance/Supplier	Final Concentration
Lim broth (Control)	Becton, Dickinson and Company	-
Human Amniotic Fluid	New England Life Sciences	2.0% (v/v)
Human Whole Blood (EDTA)	Stanford Blood Center	2.0% (v/v)
Human Whole Blood (Na Citrate)	Stanford Blood Center	2.0% (v/v)
Human Serum	Stanford Blood Center	2.0% (v/v)
Human Urine sample	In-house	2.0% (v/v)
Human Fecal sample	In-house	0.47% (w/v)
Human Meconium sample	LEE BioSolutions	1.75% (w/v)
Personal Lubricant	K-Y® Jelly Personal Lubricant (Personal Products Company, Skillman, NJ)	1.22% (w/v)
Lubricating Gel	AquaGel® Lubricating Gel (Parker Laboratories, Inc., Fairfield, NJ)	0.57% (w/v)
Vaginal Anti-itch Medication	Vagisil Cream	0.41% (w/v)
Vaginal Antifungal Medication	Monistat Cream	0.29% (w/v)
	Yeast Gard (Douche)	1.89% (w/v)
Topical Hemorrhoid Ointments	Preparation H Cream	0.26% (w/v)
Anti-Diarrheal Medications	Pepto Bismol	1.00% (w/v)
	Kaopectate	1.33% (w/v)
Deodorant Powder	Vagisil Powder	0.31% (w/v)
Deodorant Suppositories	Norforms Suppositories	0.30% (w/v)
Deodorant Spray	FDS Deodorant Spray	0.53% (w/v)
Body Powder	Gold Bond Powder	0.40% (w/v)
Body Oil	Neutrogena Body Oil	1.41% (w/v)
Spermicidal Foam	Delfen Contraceptive Foam	0.59% (w/v)
Oral Laxatives	Metamucil Fiber Supplement	0.33% (w/v)
	Exlax (Chocolate Pieces)	0.60% (w/v)
	Phillips Milk of Magnesia	1.78% (w/v)
Stool Softener	Dulcolax Suppositories	0.25% (w/v)
Enema Solution	Fleet Enema	1.93% (w/v)

g. Analytical specificity:

The analytical specificity of the Xpert GBS LB Assay was determined using a total of 100 strains representing 24 Streptococci, 76 other species including strains phylogenetically related to *S. agalactiae,* other microflora (bacteria and yeasts) commonly found in vaginal/anal flora, and human DNA. Strains were tested at concentrations of 4.5 to 9.5 x 10^8 CFU/mL. Three replicates of each strain were tested. For *Vibrio cholerae* and *Candida glabrata,* purified DNA (1500 ng/reaction)

were tested. Under the conditions of this study, all strains tested yielded correct results as "GBS Negative." The analytical specificity of the Xpert GBS LB Assay is 100%.

STRAINS TESTED		
Abiotrophia defectiva	Human DNA*	*Shigella flexneri*
Acinetobacter baumannii	*Klebsiella oxytoca*	*Shigella sonnei*
Actinobacillus pleuropneumoniae	*Lactobacillus acidophilus*	*Staphylococcus aureus*
Aeromonas hydrophila	*Lactobacillus casei*	*Staphylococcus epidermidis*
Anaerococcus lactolyticus	*Lactobacillus delbrueckii lactis*	*Staphylococcus intermedius*
Anaerococcus prevotii	*Lactobacillus gasseri*	*Staphylococcus haemolyticus*
Anaerococcus tetradius	*Lactobacillus plantarum*	*Staphylococcus lugdunensis*
Arcanobacterium pyogenes	Lactobacillus spp (CAP strain)	*Staphylococcus saprophyticus*
Bacillus cereus	*Listeria monocytogenes*	*Staphylococcus simulans*
Bacteroides fragilis	*Micrococcus luteus*	*Stenotrophomonas maltophilia*
Bifidobacterium brevis	*Moraxella atlantae*	*Streptococcus acidominimus*
Bordetella pertusis	*Moraxella catarrhalis*	*Streptococcus anginosus*
Bulkholderia cepacia	*Morganella morganii*	*Streptococcus bovis*
Candida albicans	*Neisseria gonorrhoeae*	*Streptococcus canis*
*Candida glabrata**	*Pantoea agglomerans*	*Streptococcus constellatus*
Candida tropicalis	*Pasteurella aerogenes*	*Streptococcus cricetus*
Citrobacter freundii	*Peptinophilus assacharolyticus*	*Streptococcus cristatus*
Clostridium difficile	*Peptostreptococcus anaerobius*	*Streptococcus downei*
Corynebacterium urealyticum	*Porphyromonas asaccharolytica*	*Streptococcus dysgalactiae*
Enterobacter aerogenes	*Prevotella melaninogenica*	*Streptococcus equi subsp. equi*
Enterobacter cloacae	*Prevotella oralis*	*Streptococcus gordonii*
Enterococcus durans	*Propionibacterium acnes*	*Streptococcus mitis*
Enterococcus faecalis	*Proteus mirabilis*	*Streptococcus mutans*
Enterococcus faecium	*Proteus vulgaris*	*Streptococcus oralis*
Enterococcus gallinarum	*Providencia Stuartii*	*Streptococcus parasanguinis*
Escherichia coli	*Pseudomonas aeruginosa*	*Streptococcus pneumoniae*
Finegold magna	*Pseudomonas fluorescens*	*Streptococcus pseudoporcinus*
Fusobacterium nucleatum	*Rhodococcus equi*	*Streptococcus pyogenes*
Gardnerella vaginalis	*Salmonella dublin*	*Streptococcus ratti*
Haemophilus influenzae	Human DNA*	*Vibrio cholerae**
Hafnia alvei	*Serratia marcescens*	*Yersinia enterocolitica*

*Nucleic acid was tested

h. Assay cut-off:

The valid minimum cycle threshold setting for the GBS target is 15 and the earliest GBS Ct reported from positive results during pre-clinical testing was greater than 15 cycles. The valid maximum cycle threshold setting for the GBS target is 42. To obtain a valid GBS Positive test results, the GBS Ct must be reported within the valid cycle range (Ct = 15 – 42) and the GBS fluorescent signal must be higher than the fluorescence unit threshold. To obtain a valid GBS negative test result, the GBS Ct must not be reported within the valid cycle range and the SPC Ct and IC Ct must be reported within their valid cycle range. Pre-clinical results from 235 samples were used to set these cutoffs. Assay settings were chosen to maximize the sensitivity (highest priority) and specificity of the assay. Upper and lower assay cutoffs of Ct = 15 and Ct = 42 were subsequently validated in the pivotal clinical study using receiver operator characteristics analysis.

2. Comparison studies

a. Method comparison with predicate device:

Not applicable, performance of the assay was evaluated in comparison to the gold standard/reference method (Lim broth culture).

b. Matrix comparison: Evaluation of Different Enrichment Media

Two additional enrichment broths (Trans-Vag Broth (Todd Hewitt Broth w/Gentamicin and Naladixic acid), and Strep B Carrot broth) were tested in an analytical study. GBS Serotypes II and IV were tested at 5X LoD in Lim broth, Trans-Vag broth, and Carrot broth that had been incubated with simulated background matrix for 18 hours at 35C. Negative samples consisted of background matrix only. Replicates of eight positive and negative samples were run for each broth and serotype. All positive and negative specimens were correctly identified by the Xpert GBS LB Assay. The average Ct and fluorescent values for GBS, SPC, and IC were compared for each enrichment broth tested. No statistically significant differences were observed in the analytical performance of the Xpert GBS LB assay with Lim Broth, Carrot broth, or TransVag broth media.

3. Clinical studies:

a. Prospective Clinical Studies:

Performance characteristics of the Xpert GBS LB Assay were evaluated at 3 institutions in the U.S. using the GeneXpert Dx, Infinity-48, and the Infinity-80 instrument systems. Subjects included individuals whose routine care called for collection of vaginal/rectal swab specimens for GBS testing. Prospectively collected and tested specimens consisted of excess leftover deidentified enriched Lim Broth

specimens collected for standard of care antepartum GBS testing from women at 35-37 weeks gestation.

Specimens were collected using a double swab per physician's orders for antepartum GBS testing. Swab 1 was placed in Lim broth for overnight incubation. Swab 2 used for additional testing was not related to this submission. After enrichment, an aliquot of the Lim broth was used for standard of care testing. The leftover Lim broth was used for Xpert GBS LB and for the reference culture testing.

External positive and negative quality control samples were tested daily during the clinical studies. Study specimens were not run until correct results were obtained with both external controls.

In the event of indeterminate results (INVALID, ERROR, or NO RESULT), a single retest of the Lim Broth specimen was performed. If the repeat test result was also indeterminate, it was reported as such. If the repeat test result generated a valid result, the result was reported.

The Xpert GBS LB Assay performance was compared to the reference culture procedure consisting of Lim Broth enrichment of the vaginal/rectal swab by incubating for 18 to 24 hours at 35-37ºC. Enriched Lim broths were then subcultured to 5% sheep blood agar plates and incubated for 18-24 hours at 35-37ºC with 5% CO_2. Plates with no GBS 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 by latex agglutination.

A total of 826 specimens meeting the predetermined inclusion and exclusion criteria were tested for GBS by the Xpert GBS LB Assay and culture. Xpert GBS LB Assays for 98.1% (810/826) of eligible specimens were successful on the first attempt. The indeterminate cases included seven ERROR results, two INVALID results, and two NO RESULT outcomes. All of the 16 indeterminate cases were retested and yielded valid results upon repeat assay. The overall rate of assay success was 100% (826/826).

The Xpert GBS LB Assay demonstrated an overall sensitivity and specificity for detection GBS colonization of 99.0% and 92.4%, respectively, relative to Lim broth culture.

Xpert Assay

		Reference Culture Method - Lim broth culture		
Xpert GBS		Pos	Neg	Total
	Pos	189	48[a]	237
	Neg	2[b]	587	589
	Total	191	635	826
		Sensitivity:	99.0% (95% CI: 96.3-99.9)	
		Specificity:	92.4% (95% CI: 90.1-94.4)	
		PPV:	79.7% (95% CI: 74.1-84.7)	
		NPV:	99.7% (95% CI: 98.8-100)	

GBS LB Overall

Performance vs. Culture

[a]Testing result by sequencing: 47 of 48 GBS specimens were sequenced, 42 were GBS positive, 5 were GBS negative and one Lim broth was not sequenced.
[b]Testing result by sequencing: 2 of 2 GBS specimens were sequenced, both were GBS negative.

Bi-directional sequencing of Lim broth specimens using different primers than those in the Xpert GBS LB assay was performed on specimens with discrepant results. Of the specimens that yielded a false positive result with the Xpert GBS LB assay as compared to the reference culture, 47/48 specimens were tested by bi-directional sequencing. Testing resulted in 42 GBS positive and 5 GBS negative. One discrepant specimen was not tested. Two specimens that yielded false negative results with the Xpert GBS LB assay as compared to the reference culture were also tested by bi-directional sequencing. Both specimens yielded a negative result. Results of the additional testing are presented as additional supportive information as footnotes under the performance table above and in the package insert.

b. Other clinical supportive data

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

Approximately 10-30% of pregnant women are colonized with GBS in the vagina or rectum. GBS colonization can be transient, chronic, or intermittent. Culture screening of both the vagina and rectum for GBS late in gestation during prenatal care can detect

women who are likely to be colonized with GBS at the time of delivery. During the clinical evaluation for the Xpert GBS LB Assay, 28.7% (237/826) of women were colonized with GBS by Xpert GBS LB Assay.

N. Instrument Name:

GeneXpert Instrument Systems

- GeneXpert Dx System
- GeneXpert Infinity-48 System
- GeneXpert Infinity-80 System

O. System Descriptions:

1. Modes of Operation:

   The GeneXpert Instrument System family comprises a GeneXpert (GX) instrument (GX-I, GX-IV, GX-XVI), a GeneXpert Infinity-48 instrument, or a GeneXpert Infinity-80 instrument; a computer and preloaded software for running tests and viewing the results. The GX-I contains 1 module, the GX-IV contains 1-4 modules, and the GX-XVI contains up to four banks of 4 modules and is available to the customer in configurations of 4, 8, 12 or 16 modules. The GeneXpert Infinity-48 contains up to 6 banks of 8 modules and is available in configurations of 16, 24, 32, 40 and 48 modules. The GeneXpert Infinity-80 contains up to 10 banks of 8 modules and is available in configurations of 16, 24, 32, 40, 48, 56, 64, 72 or 80 modules. Further, the GeneXpert Dx Systems can be supplied with either a laptop computer or a desktop computer. Each of the GeneXpert Instrument Systems process data in the same manner, *i.e.*, using the same optics, calibration and signal processing.

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:
   To perform a test, the user selects the 'Create Test' or 'Orders' icon, scans the cartridge barcode, enters or scans the sample ID barcode, and loads the cartridge into the module (for the GeneXpert Dx System) or onto the conveyor (for the GeneXpert Infinity Systems) to start the test. The specimen is identified by the instrument using information encoded in the consumable.

5. Calibration:

Optical and thermal calibration of the GeneXpert Instrument Systems is performed at the time of manufacture, prior to installation. Further calibrations are performed by Cepheid and are recommended yearly or every 2,000 tests per instrument module. The user does not calibrate or perform any serviceable functions on the instruments.

6. Quality Control:

   The integrity of the system is verified and controlled by specific hardware/software checks during the cartridge load process, and during the test itself. These checks, in combination with the assay internal controls, are employed to monitor the performance of the system during operation and alert the user if an out of specification condition exists. See description of internal and external control testing in section "M" above.

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.