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

A. 510(k) Number: k060540

B. Purpose for Submission: New 510k

C. Measurand: Group B Streptococcus (*S. agalactiae*) DNA (3" region adjacent to *cfb* gene)

D. Type of Test: Nucleic acid amplification assay system, automated

E. Applicant: Cepheid

F. Proprietary and Established Names: XPert GBS™ and Nucleic acid amplification assay system, Group B Streptococcus, direct specimen

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3740

2. Classification: Class I

3. Product code: NJR

4. Panel: 83

H. Intended Use:

1. Intended use(s):

The Cepheid Xpert GBS performed on the GeneXpert Dx System is a qualitative *in vitro* diagnostic test designed to detect Group B Streptococcus (GBS) DNA from vaginal/rectal swab specimens, using fully automated real-time polymerase chain reaction (PCR) with fluorogenic detection of the amplified DNA.2. Indication(s) for use:

2. Indications for use:
Xpert GBS Assay testing is indicated for rapid identification of antepartum and intrapartum GBS colonization.

- The use of the Xpert GBS for intrapartum screening should not preclude the use of other strategies (e.g., antepartum testing). Intrapartum Xpert GBS results are useful to identify candidates for intrapartum antibiotic prophylaxis when administration of intravenous antibiotics is not delayed pending results.

- The Xpert GBS 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

4. Special instrument requirements: GeneXpert Dx System (instrument, computer, barcode wand reader)

I. Device Description:

The Xpert GBS™ is a single-use cartridge with pre-packaged reagents for detection of GBS DNA. After insertion of the specimen swab and provided reagents, the cartridge is processed by the GeneXpert Dx System instrument. The cartridge contains an internal control and a system control (probe check). Testing is completed in ~75 min. with results displayed in tabular and graphic formats.

Each cartridge has 11 separate chambers, a syringe with plunger, a rotary valve for controlling fluid movement, a filtration area for capturing, concentrating, washing and lysing, dry real-time PCR reagents (primers and probe), an integrated PCR reaction tube that is automatically filled by the instrument.

During sample preparation, bacterial cells are captured on a filter, cells are lysed by an ultrasonic horn, and DNA is eluted. The eluate rehydrates dry PCR reagents. The reaction mixture is transferred to a reaction tube for real-time PCR, alternate thermalcycling and fluorescent detection. Dried PCR reagents (polymerase/inhibitor complex, primers for target and controls, Taqman probes for target and controls), dNTPs, internal DNA control are freeze-dried on beads within each cartridge. A sample processing control, *B. globigii* (~2000 spores) is also freeze-dried on a bead contained within the cartridge.

The sample processing control (SPC) ensures correct processing of each sample by monitoring the lysis and elution processing. The SPC may not amplify in a high-positive sample. An internal control (IC), comprised of approximately 2250 copies of a linearized plasmid containing a 212 base pair chimeric construct (T. foetus /Y. enterocolitica), verifies functional PCR reagents and the absence of inhibition that would prevent PCR amplification. A probe check feature built into the GeneXpert instrument function before the start of the PCR reaction, measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability.

The GenXpert Dx System instrument has 4 randomly accessible modules. Additionally a barcode wand reader can be used to identify and track specimen IDs and results. Copan Venturi Transystem Culture Swab and Transport System is used for specimen collection.

Xpert GBS™ Reagent Kit

GeneXpert Dx System

J. Substantial Equivalence Information:

1. Predicate device name(s): IDI-Strep B Assay
2. Predicate 510(k) number(s): k022504
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Rapid identification of Group B Streptococcus	Rapid identification of Group B Streptococcus
Samples	vaginal/rectal swab specimens from ante-partum and intra-partum women	vaginal/rectal swab specimens from ante-partum and intra-partum women
Indications	Identification of GBS colonization	Identification of GBS colonization
Technological Principles	Nucleic acid amplification (DNA); real-time PCR	Nucleic acid amplification (DNA); real-time PCR

Differences		
Item	Device	Predicate
DNA target sequence	3' region adjacent to *cfp* gene	*cfp* gene
Instrumentation	GeneXpert automates sample processing with real-time PCR	SmartCycler for real-time PCR; manual sample processing
Lysis	Sonication	Glass beads
Internal Controls	Sample processing control (SPC), Internal control (IC), Probe check	Internal control (IC)
Test time	~75 min	~60 min

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

L. Test Principle:

The Xpert GBS Test uses real-time PCR adapted to an automated instrument system that integrates sample processing (lysis and DNA purification) in a cartridge that contains freeze-dried PCR reagents and process controls for real-time PCR detection. This integration of nucleic acid extraction reduces labor-intensive steps requiring specially trained personnel and also reduces likelihood of cross-contamination and technician-dependent variability. Sonication, integrating ultrasonic energy and glass beads, mechanically lyses cells from specimen material.

The Xpert GBS Target region is a 3' untranslated region of the *cfb* gene. Subsequent to identification of target sequences, a report suggests that the target is an open reading frame adjacent to, rather than the *cfb* gene itself. The resulting amplicon is 132 bp. Probes are labeled with FAM (5') and QSY7 quencher (3'). Note: Primers are 23 mer (Tm 72.6. 52.2% GC) and 9 mer (70.0 Tm, 57.9 %GC) while the GBS probe is 27 mer (73.5 Tm, 44.4 %GC). Cycling parameters are 3 min at 95º (denaturation), followed by 45 cycles of 5 s at 95º, 20s at 60º, and 18 s 73º.

The GenXpert® instrument has 4 modules, each with an I-Core for thermal cycling and real-time detection of PCR amplicons. Each of the 4 modules is controlled independently, allowing for specimens to be loaded and tested when received, rather than batching. Each of the 4 modules in addition to the I-Core, has a syringe drive for aspirating and dispensing fluids, a valve drive for chamber access, and an ultrasonic horn for lysis. Prior to operation, a self-test verifies heater, fan and optics functionality, while the syringe drive, valve and ultrasonic horn current are continuously checked.The GenXpert can simultaneously detect signal from up four different spectral bands.

Each I-core has two ceramic plates to assure temperature uniformity and rapid heat transfer. Firmware controls the temperature inside by moving ambient air across the heater plates. Optics consist of a 4-color excitor module (high intensity LEDs to excite reporter dye molecules) and a 4-color detector module (silicon photodetectors and filters to detect the 4 spectral bands). Calibration and data analysis algorithms compensate for spectral overlap. Thermal reaction chamber thermistors are calibrated to $\pm$0.50ºC and calibration coefficients correct for errors in raw thermistor reads (stored in firmware). Individual dye-oligos signals are corrected using spectral characteristics of pure dye-oligos after subtracting raw signal produced by a reaction tube alone.

For the GBS application, three spectral signals are processed (separate dyes tagged to oligonuclide probes for the SPC, IC, and the GBS). Two of the 3 signals are from fixed components within the reagents, with one from unknown. Output results are color coded (red-positive; green-negative; gray-invalid; gold-error); tabular and graphic formats are accessible by password. Setup requires a surge protector and printer. A laptop preloaded with system and other required software is provided (also power cord, ethernet cable, and barcode scanner).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:
   A panel of specimens with varying concentrations of GBS and *Lactobacillus acidophilus* (negative) were tested in triplicate on 10 different days at each of the three sites (4 specimens × 3 × 10 days × 3 sites). One lot of Xpert GBS kits was used at each of the 3 testing sites, according to the Xpert GBS procedure. All 360 Xpert GBS tests with all samples yielded results that agreed with expected results.

Table 7: Summary of Reproducibility Results.

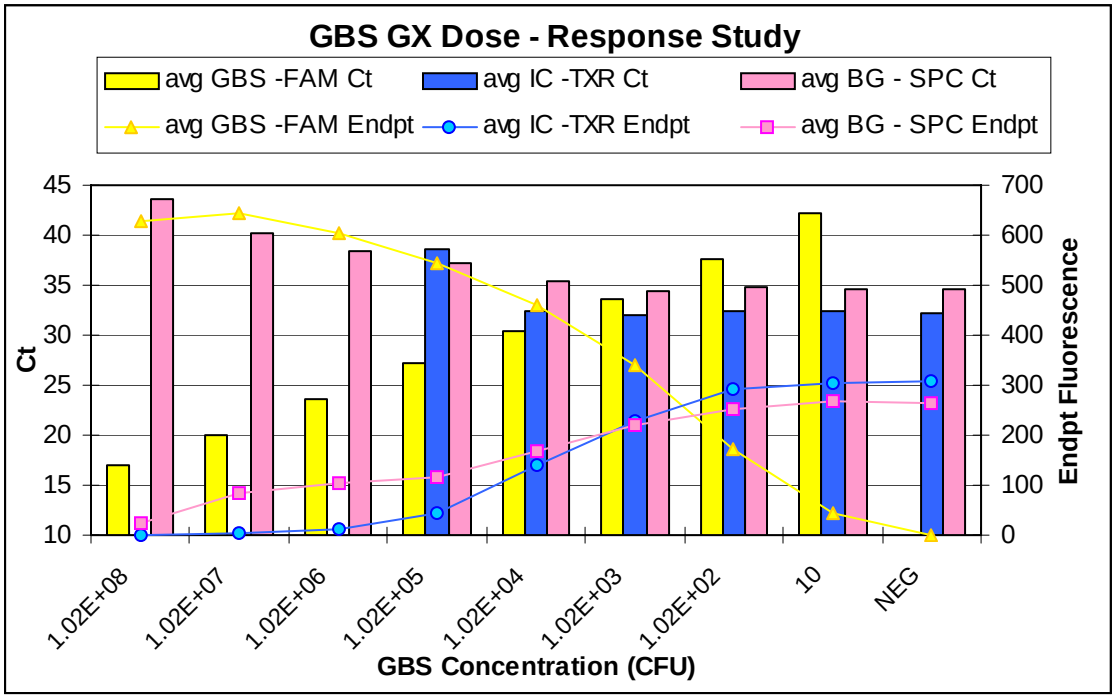
Sample CFU/swab	Site 1	Site 2	Site 3	Expected Results (Ct range)1	Total Agreement	Total % Agreement
GBS Negative	30/30	30/30	30/30	Negative (0, or >42)	90/90	100%
L. acidophilus 1.7 × 10^4 CFU /swab						

GBS low 6.2 × 10^2 CFU/swab	30/30	30/30	30/30	Positive (31 to 41)	90/90	100%
GBS moderate 8.3 × 10^3 CFU/swab	30/30	30/30	30/30	Positive (27 to 37)	90/90	100%
GBS high 1.3 × 10^6 CFU/swab	30/30	30/30	30/30	Positive (19 to 29)	90/90	100%
Total Agreement	120/120	120/120	120/120		360/360	100%
% Agreement	100%	100%	100%		100%	100%

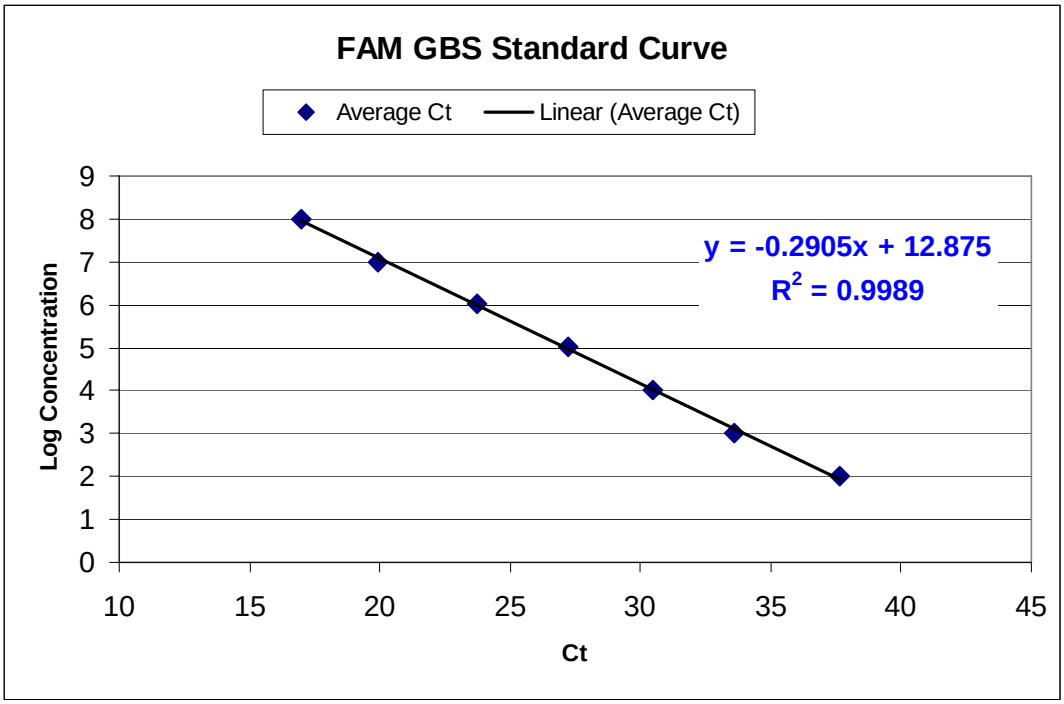
[1]Expected range of Ct values; all values were within expected ranges.

b. *Linearity/assay reportable range:*
A stock concentration of GBS (1.36e9 CFU/*mL*) was serially diluted over 8 logs and loaded directly into each cartridge with appropriate sample preparation reagents. Three replicates at each dilution were tested. Results demonstrated linearity (r^2=0.9989) of GBS detection (FAM) as a function of GBS cell input over 7 logs. The reported Ct range was 17.0 – 37.7 in this study. See figures below.

Mean Cycle Threshold and Endpoint Fluorescence Values



Dynamic Range Standard Curve

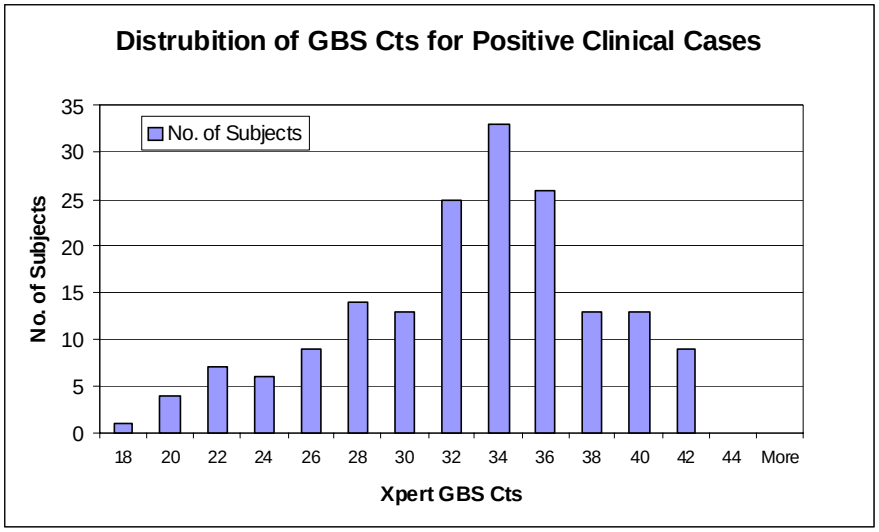


c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Recommended external controls for testing contain approximately 100 CFU/swab and should yield approximately a 34 Ct

d. Detection limit:
The analytical sensitivity, or limit of detection (LOD), was determined using 11 *S. agalactiae* strains. Nine distinct GBS serotypes have been identified (Ia, Ib, II, III, IV, V, VII, and VIII). Most cases of neonatal sepsis caused by GBS are attributed to 1 of 4 serotypes: Ia, Ib, II, or III. GBS type V has emerged as an important cause of GBS infection in the United States, and strains of types VI and VIII have become prevalent among Japanese women.9 Quantitated cultures were tested in four replicates. The lowest concentration of each subtype resulting in a positive result in all four replicates was 250 CFU/swab.

e. Analytical specificity:
Commercially obtained, purified genomic DNA from 101 strains representing 28 Streptococci, 73 other species including strains phylogenetically related to *S. agalactiae*, other microflora (bacteria and yeasts) commonly found in vaginal and anal flora, and human DNA were tested. Replicates of three were tested at 1.5 ng/25 µl reaction (~2 × 105 equivalent genome copies per reaction). None of the 28 Streptococcal isolates (non-GBS) tested positive. Of the remaining 73 strains, four (*Enterococcus gallinarium*, *Staphylococcus simulans*, *Micrococcus luteus*, and *Propionibacterium acnes*) were weakly positive in one of six replicates.



f. Assay cut-off:

During analytical and pre-clinical studies with GBS Xpert, the various acceptable ranges for SPC and the IC were verified:

	Valid Ct Range	LSP negatives	FAC*	Endogenous substances	Pre-Clinical negatives
		(n=160)	(n=80)	(n=37)	(n=181)
CIC	28-38				
mean		32.0	32.5	32.2	31.5
sd		0.5	1.3	1.0	0.4
min		29.5	28.2	31.1	29.4
max		33.4	41.4	35.9	33.6
SPC	32-41				
mean		35.0	35.1	36.0	35.8
sd		0.8	0.7	0.9	1.4
min		33.7	33.9	34.9	33.1
max		38.3	37.4	39.6	40.5

(*) FAC testing is done in the presence of 0.02 % ferric ammonium citrate and GBS at 1000 CFUs per sample.

The valid cycle range for GBS was set at 15-42. during clinical studies, the majority of Xpert GBS positive specimens had Cts from 28-40 (see histogram below).

2. Comparison studies:

a. Method comparison with predicate device:
During the clinical evaluations of Xpert GBS, one swab was used in the Xpert GBS Assay on the GeneXpert Dx System; the other was used in the predicate NAAT assay. The predicate NAAT assay targets a sequence in the *cfb* gene and was previously FDA-cleared. Results from this test were not used in performance estimations.

	Culture Pos			Culture Neg			Culture ND			
	2nd NAAT Pos	2nd NAAT Neg	Unresolved	2nd NAAT Pos	2nd NAAT Neg	Unresolved	2nd NAAT Pos	2nd NAAT Neg	Unresolved	Total Xpert GBS
Xpert GBS Pos	149	27	2	10	9	0	0	0	0	197
Xpert GBS Neg	6	17	0	11	549	1	0	3	0	587
Invalid/Error/No Result	0	0	0	1	9	0	0	0	0	10
Total 2nd NAAT	165	44	2	22	567	1	0	3	0	794

	Xpert GBS						2nd NAAT					
Category	Sensitivity	Lower CI	Upper CI	Specificity	Lower CI	Upper CI	Sensitivity	Lower CI	Upper CI	Specificity	Lower CI	Upper CI
Overall	88.6% (178/201)	83.3%	92.6%	96.7% (561/580)	94.9%	98.0%	77.9% (155/199)	71.5%	83.5%	96.3% (567/589)	94.4%	97.6%
Antepartum	85.3% (87/102)	76.9%	91.5%	98.1% (259/264)	95.6%	99.4%	74.5% (76/102)	64.9%	82.6%	97.0% (263/271)	94.3%	98.7%
Intrapartum	91.9% (91/99)	84.7%	96.4%	95.6% (302/316)	92.7%	97.6%	81.4% (79/97)	72.3%	88.6%	95.6% (304/318)	92.7%	97.6%
ROM[2]	91.3% (21/23)	72.0%	98.9%	94.3% (100/106)	88.1%	97.9%	90.9% (20/22)	70.8%	98.9%	95.2% (100/105)	89.2%	98.4%
No ROM[3]	92.1% (70/76)	83.6%	97.0%	96.2% (202/210)	92.6%	98.3%	78.7% 59/75)	67.7%	87.3%	95.8% (204/213)	92.1%	98.0%

[1] The 2nd NAAT targets a sequence in the *cfb* gene and was previously FDA-cleared.
[2] Subset of intrapartum women who had specimens collected after membrane rupture (rupture of membrane, ROM).
[3] Subset of intrapartum women who had specimens collected before membrane rupture.

b. Matrix comparison: NA

3. Clinical studies:

The Xpert GBS Assay was evaluated at six institutions with maternity services in the United States. Each institution had a culture-based or nucleic acid amplification test (NAAT) based screening program. Testing was done in clinical laboratories affiliated with each institution as well as the labor and delivery area. Both intrapartum and antepartum subjects were included in the study. To be enrolled in the intrapartum portion of the study, women had to provide written consent, be in labor, and have no contraindication to vaginal examination (for example, bleeding). To be enrolled in the antepartum portion of the study, women had to provide written consent, be at 35-37 weeks gestation, and have no contraindication to vaginal examination (for example, bleeding). There was also no evidence of placenta previa, no urgent indication to proceed to delivery, and no antibiotic used in the week prior to admission for all subjects.

Vaginal/rectal specimens were collected from each of 794 eligible subjects using two sets of marked swabs (Cepheid GBS Collection Devices). One of the swabs from the first set was used for culturing. The second set of marked swabs was divided: one swab was used in the

Xpert GBS Assay on the GeneXpert Dx System; the other was used in the 2nd NAAT assay. The 2nd NAAT assay targets a sequence in the cfb gene and was previously FDA-cleared. Results from this test were not used in performance estimations. After use in these tests, each of these swabs was also placed into LIM culture broth, incubated, subcultured onto blood agar plate (BAP) and observed for GBS.

Each institution used the culture technique recommended in the 2002 CDC guidelines: microbiological culture in selective broth medium (LIM broth), followed by 18–24 h incubation, and subculture onto BAP. Colonies consistent with GBS were specifically identified by slide agglutination testing.

The performance of the Xpert GBS Assay relative to GBS status determined from laboratory cultures of specimens from 794 maternity patients: 373 antepartum and 421 intrapartum. Three women had no results by culture and were excluded from analyses (0 were Xpert GBS positive and 3 were Xpert GBS negative), leaving 791 patients that could be evaluated. All subjects had culturing done (as described above) and most also had a 2nd GBS NAAT performed. The 2nd NAAT targets a sequence in the cfb gene and was previously FDA-cleared; results from this test were not used in performance estimations. Of the 791 cases, the Xpert GBS assay yielded 726 reportable results on the first attempt (91.8%). There were 65 non-reportable results (i.e., invalid, error, or no result); 55 of these cases resolved upon repeat testing. Overall, 201 women had cultures positive for GBS, either from the single swab used for culture or the eluted swabs from Xpert GBS and 2nd NAAT testing. The Xpert GBS assay yielded 168 positive results initially (168/201, 83.6%). After repeat testing, the positive results increased to 178/201, or 88.6%. 590 women had negative cultures and 520 were negative initially with Xpert GBS testing (88.1%), and 561 after repeat testing (95.1%).

The table below shows performance of Xpert GBS testing compared to positive and negative GBS culture findings for 791 subjects (3 subjects had cultures that were overgrown or could not be otherwise interpreted). Sensitivity, specificity, and negative and positive value estimates shown are based on results after repeat testing.

Patient Category	Results	Total N[1]	Culture Positive Patients[1]	Culture Negative Patients[1]	Sensitivity after repeat testing [95% confidence]	Specificity after repeat testing [95% confidence]	PPV[7] after repeat testing [95% confidence]	NPV[8] after repeat testing [95% confidence]
All Patients	Xpert GBS Pos	197 (186)	178 (168)	19 (18)	88.6%	96.7%	90.4%	96.1%
	Xpert GBS Neg	584 (540)	23 (20)	561 (520)	[83.3%-92.6%]	[94.9%-98.0%]	[85.4%-94. 1%]	[94.2%-97.5%]
	No Result[2]	10 (65)	0 (13)	10 (52)				
	Total	791[3]	201[4]	590				
Antepartum	Xpert GBS Pos	92 (88)	87 (83)	5 (5)	85.3%	98.1%	94.6%	94.5%
	Xpert GBS Neg	274 (253)	15 (13)	259 (240)	[76.9%-91.5%]	[95.6%-99.4%]	[87.8%-98.2%]	[94.1%-96.9%]
	No Result[2]	7 (32)	0 (6)	7 (26)				
	Total	373	102	271				
Intrapartum	Xpert GBS Pos	105 (98)	91 (85)	14 (13)	91.9%	95.6%	86.7%	97.4%
	Xpert GBS Neg	310 (287)	8 (7)	302 (280)	[84.7%-96.5%]	[92.7%-97.6%]	[78.6%-92.5%]	[95.0%-98.9%]
	No Result[2]	3 (33)	0 (7)	3 (26)				
	Total	418	99	319				
ROM[5]	Xpert GBS Pos	27 (24)	21 (19)	6 (5)	91.3%	94.3%	77.8%	98.0%
	Xpert GBS Neg	102 (92)	2 (2)	100 (90)	[72.0%-98.9%]	[88.1%-97.9%]	[57.7%-91.4%]	[93.1%-99.8%]
	No Result[2]	0 (13)	0 (2)	0 (11)				
	Total	129	23	106				
No ROM[6]	Xpert GBS Pos	78 (74)	70 (66)	8 (8)	92.1%	96.2%	89.7%	97.1%
	Xpert GBS Neg	208 (195)	6 (5)	202 (190)	[83.6%-97.1%]	(92.6%-98.3%]	(80.8%-95.5%]	(93.8%-98.9%]
	No Result[2]	3 (20)	0 (5)	3 (15)				
	Total	289	76	213				

[1] All Xpert GBS results are shown after repeat testing.
[2] 'No results' from an Xpert GBS test could be due to an invalid test, a system error, or a no result when the presence or absence of GBS DNA could not be reported.
[3] Three intrapartum women with no results by culture are excluded from the analyses.
[4] Overall prevalence of GBS colonization as determined by culture is 25.3%.
[5] The subset of intrapartum women who had specimens collected after membrane rupture (rupture of membrane, ROM).
[6] The subset of intrapartum women who had specimens collected before membrane rupture. There would be no biological differences expected between these intrapartum specimens and those collected antepartum.
[7] Positive predictive value.
[8] Negative predictive value.

b. Clinical specificity: see above

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

4. Clinical cut-off: NA

5. Expected values/Reference range:
Approximately 10–30% of pregnant women are colonized with GBS in the vagina and/or rectum. GBS colonization can be transient, chronic, or intermittent. During clinical evaluations for the Xpert GBS assay, 25.4% (201 of 791) women (antepartum and intrapartum) were colonized with GBS by culture methods.

N. Instrument Name: GeneXpert Dx system

O. System Descriptions:

1. Modes of Operation: 4 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests.

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: barcodes

4. Specimen Sampling and Handling: automated

GeneXpert Dx System Hardware Components for Automated Sample Processing

Module Hardware Components	Function
Valve Drive	Rotates the cartridge valve body to address the different cartridge chambers.
Syringe Pump drive	Dispenses fluids to and from the different cartridge chambers.
Ultrasonic horn	Lyses the bacterial cells and sample prep control.
I-CORE® module	Performs PCR amplification and detection. As the user inserts the cartridge into the system, the reaction tube component of the cartridge is inserted into the I-CORE module. After sample preparation within the cartridge, the sample and reagent mixture is transferred from the cartridge chamber into the reaction tube. During the amplification process, the I-CORE heater heats up and the fan cools down the reaction tube contents. Two optical blocks positioned within the I-CORE excite the dye molecules that make up the probes and detect the fluorescence emitted. The system uses calibration and data analysis algorithms to determine a relative fluorescence value for each reporter dye after each thermal cycle.

5. Calibration: Optical and thermal calibration of the GeneXpert Dx System is performed by Cepheid at the time of manufacture prior to installation and once yearly or after 1000 runs per module. The user does not calibrate or perform any serviceable functions on the instrument. The normalization function compensates for any optical degradation between calibrations.

The thermal reaction chamber thermistors are calibrated to ± 0.50ºC using National Institute of Standards and Technology (NIST)-traceable standards. During the manufacturing process, the temperature of the heating system is measured at two temperatures: 60ºC and 95ºC. Calibration coefficients that correct for small errors in the raw thermistor readings of the heaters are stored in the memory of each I-CORE module.

The optical system is calibrated using standard concentrations of individual unquenched fluorescent dye-oligos. For each optical channel, the signal produced by a tube alone (the blank signal) is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

6. Quality Control:

Before the start of the PCR reaction, the GeneXpert Dx System is programmed to perform a probe check on the GBS target, CIC and SPC. The Probe Check control verifies reagent bead rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. PC is considered to PASS if it meets the predetermined acceptance criteria. If the PC fails in GBS target, CIC or SPC; the test will not continue.

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

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.