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

I Background Information:

A 510(k) Number

K203429

B Applicant

Cepheid

C Proprietary and Established Names

Xpert GBS LB XC, GeneXpert Dx System, GeneXpert Infinity System

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

To obtain substantial equivalence determination for the Xpert GBS LB XC

B Measurand:

Two conserved regions within the *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) genome:

- Coding region for a glycosyl transferase family protein
- Coding region for a LysR family transcriptional regulator

C Type of Test:

Real Time Polymerase Chain Reaction (PCR)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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

Xpert GBS LB XC testing is indicated as an aid in determining the GBS colonization status of antepartum women.

- The Xpert GBS LB XC test is intended for antepartum testing on enriched Lim broth cultures of vaginal/rectal swabs after 18–24 hours of incubation
- The Xpert GBS LB XC test does not provide antimicrobial susceptibility test results. Culture is necessary to obtain isolates to perform susceptibility testing as recommended for penicillin-allergic women.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Erroneous test results might occur from improper specimen collection, handling or storage, technical error, or sample mix-up. Careful compliance to the instructions in this insert is important to avoid erroneous results.

D Special Instrument Requirements:

GeneXpert Instrument Systems

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

IV Device/System Characteristics:

A Device Description:

The Xpert GBS LB XC test is an automated *in vitro* diagnostic test for qualitative detection of DNA from Group B *Streptococcus* (GBS) from vaginal/rectal swab specimens after enrichment in Lim broth (LB). The test is performed on the GeneXpert Instrument System which automates sample preparation, nucleic acid amplification and real-time detection of target sequences. The system consists of an instrument, computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use, disposable, multi-chambered

fluidic cartridges (Xpert GBS LB XC cartridges) that are designed to complete sample preparation and real-time PCR for the detection of GBS genomic DNA in ~24 minutes or less.

The primers and probes in the Xpert GBS LB XC test are designed to simultaneously amplify and detect two unique GBS chromosomal targets: the first is a target within a coding region for a glycosyl transferase family protein and the second is within a coding region for a LysR family transcriptional regulator of *Streptococcus agalactiae* DNA. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate extraction and processing of the target sequences and to monitor for the presence of inhibitors in the PCR reaction. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

B Principle of Operation:

The GeneXpert Instrument System, comprised of the GeneXpert Dx, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems, have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR tests. Each GeneXpert module is identical and operates independently. Each module consists of a syringe pump drive for dispensing fluids, an ultrasonic horn, a valve drive to rotate the cartridge valve body to address the different cartridge chambers for sample movement, and proprietary microprocessor controlled I-CORE (Intelligent Cooling/Heating Optical Reaction) – thermocycler for performing real-time PCR amplification and fluorescence signal detection.

All systems are designed to use assay-specific, single-use disposable cartridges that hold the PCR reagents and host the PCR process. The single-use cartridges contain: (1) eleven chambers for holding sample, reagents, or other materials, (2) a valve body composed of a plunger and syringe barrel, (3) a rotary valve system for controlling the movement of fluids between chambers, (4) an area for capturing, concentrating, washing, and lysing cells, (5) dry real-time PCR reagents, (6) an integrated PCR reaction tube that can be automatically filled by the instrument, and (7) liquid reagents. To minimize test-to-test contamination, all fluids including amplicons and waste materials are contained within the cartridge. The instrument does not come into contact with any fluids within the cartridge. Each disposable cartridge is intended to test one sample.

After collecting and transporting a swab specimen to the laboratory, the swab is placed in Lim broth for enrichment overnight at 35–37°C. Post-enrichment, a clean swab is dipped into the Lim broth which is then transferred to the sample chamber of the cartridge. The Xpert GBS LB XC cartridge is loaded on the GeneXpert Instrument System platform, which performs hands-off, automated sample processing and real-time PCR for detection of bacterial DNA. The sample results are interpreted by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms.

C Instrument Description Information:

1. Instrument Name:

   The Xpert GBS LB XC test can be run on the following GeneXpert Instruments:
   - GeneXpert Dx (software version 6.2)

- GeneXpert Infinity-48s (software version Xpertise 6.8)
- GeneXpert Infinity-80 (software version Xpertise 6.8)

2. Specimen Identification:

   To perform a test with the GeneXpert System, the user selects the 'Create Test' (GeneXpert Dx) or 'Orders' and 'Order Test' (GeneXpert Infinity) icon, types or scans the patient/sample ID barcode, scans the cartridge 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.

3. Specimen Sampling and Handling:

   After overnight enrichment of the swab specimen in Lim broth, a clean swab is dipped into the sample and then transferred to the sample chamber of the cartridge which is loaded on the GeneXpert Instrument System platform for automated sample processing and real time PCR for detection of targets.

4. Calibration:

   Sample processing control (SPC): Ensures the sample was correctly processed. The SPC is *B. globigii* in the form of a dry bead and is included in each cartridge. The SPC monitors the lysis and elution processing. The SPC passes if it meets the assigned acceptance criteria. If the SPC fails, the test result will be INVALID and the specimen should be retested.

   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. The Probe Check passes if it meets the assigned acceptance criteria. If the PCC fails, a probe check ERROR is reported, and the specimen should be retested.

5. Quality Control:

   Commercially available external positive (inactivated *S. agalactiae*) and negative (inactivated *L. acidophilus*) controls were run each day of the clinical study prior to testing study samples. External controls came in ready-to-use vials such that sterile swabs were submerged into the vials and then inserted into the Xpert GBS LB XC cartridge to be tested according to the package insert. If an external control result was indeterminate or incorrect, the external control was repeated until the correct result was obtained. A total of 47 positive and 47 negative control results were obtained in the clinical study. Two samples generated an indeterminate result; one intermediate result was resolved after the external control sample was retested once and the other intermediate result was resolved after the external control sample was retested twice.

   External controls may be used in accordance with local, state, or federal accrediting organizations, as applicable.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Xpert Gbs Lb Genexpert Dx Systems (gx-i, Gx-iv) Genexpert Dx Systems (gx-xvi) Genexpert Infinity-48 System (900-xxxx)

B Predicate 510(k) Number(s):
K121539

C Comparison with Predicate(s):

Device & Predicate Device(s):	New Device K203429	Predicate Device K121539
Device Trade Name	Xpert GBS LB XC	Xpert GBS LB Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Xpert GBS LB XC test, performed on the GeneXpert Instrument Systems, is an automated qualitative in vitro diagnostic test for the detection of Group B *Streptococcus* (GBS) DNA from enriched vaginal/rectal swab specimens, using real- time polymerase chain reaction (PCR). Xpert GBS LB XC testing is indicated as an aid in determining the GBS colonization status of antepartum women. • The Xpert GBS LB XC test is intended for antepartum testing on enriched Lim broth cultures of vaginal/rectal swabs after 18–24 hours of incubation • The Xpert GBS LB XC test does not provide antimicrobial susceptibility test results. Culture is necessary to obtain isolates to perform susceptibility testing as recommended for penicillin-allergic women.	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.

Device & Predicate Device(s):	New Device K203429	Predicate Device K121539
Organism Detection	Group B *Streptococcus*	Same
Instrument Systems	GeneXpert Dx System, GeneXpert Infinity-48 System, GeneXpert Infinity-80 System	Same
Specimen Type	Enriched Lim broth cultures from vaginal/rectal swabs after 18-24 hours of incubation	Same
Collection and Transport Media	Cepheid Collection Device or swab in a non-nutritive transport medium	Same
Assay Technology	Real-Time PCR	Same
Self-Contained System Assay	Yes	Same
Single Use	Yes	Same
Automated extraction, detection and result interpretation	Yes	Same
Fluidics	Self-contained	Same
External Assay Controls	Materials available, but not required	Same
Built in Lysis Control	Yes	Same
Assay Results	Qualitative	Same
Time to Result		≤ 55 minutes total after sample addition to cartridge
General Device Characteristic Differences		
DNA Target Sequence	Two conserved chromosomal sequences in *S. agalactiae:* • Coding region for a glycosyl transferase family protein • Coding region for a LysR family transcriptional regulator	3′ untranslated region of the *cfb* gene of *S. agalactiae*
Assay Internal Controls	Integrated specimen processing control and probe check control	Integrated internal process control, specimen processing control and probe check control
Early Assay Termination Feature	Yes	No

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3 *Evaluation of Precision Performance of Quantitative Measurement Methods*; Approved Guideline – Third Edition
- CLSI EP07-A3 *Interference Testing in Clinical Chemistry*; Approved Guideline – Third Edition.
- CLSI EP09-A3 *Measurement Procedure Comparison and Bias Estimation Using Patient Samples*; Approved Guideline – Third Edition
- CLSI EP15-A3 *User Verification of Performance for Precision and Trueness*; Approved Guideline – Third Edition.
- CLSI EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*; Approved Guideline – Second Edition.
- CLSI MM03 *Molecular Diagnostic Methods for Infectious Disease*; Approved Guideline – Third Edition.
- CLSI MM13-A *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods*; Approved Guideline.
- CLSI EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents*; Approved Guideline.
- ISO 14971:2019 Medical devices — *Application of risk management to medical devices*
- BS EN ISO 23640 *In vitro diagnostic medical devices. Evaluation of Stability Testing of in vitro Diagnostic Reagents*, June 2015
- *Guidance for Industry and FDA Staff – General Principles of Software Validation*, issued January 11, 2002.
- *Guidance for Industry and FDA Staff – Format for Traditional and Abbreviated 510(k)*, issued August 12, 2005.
- *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 Industry and FDA Staff – Content of Premarket Submissions for Management of Cybersecurity in Medical Devices*, issued on October 2, 2014.
- *Guidance for Industry - Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software*, issued January 14, 2005.
- *Guidance for Industry and FDA Staff – Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*, issued May 11, 2005.
- *Guidance for Industry, FDA Reviewers and Compliance on Guidance for Off-the-Shelf Software Use in Medical Devices*; issued September 9, 1999.
- *Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff - Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable*, issued April 25, 2006.
- FDA QSR 21 CFR 820: Quality System Regulation
- ISO 13485:2016 SO 13485:2016 *Medical devices — Quality management systems — Requirements for regulatory purposes*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

To evaluate the reproducibility of the Xpert GBS LB XC, a panel of ten samples consisting of three different hemolytic Group B *Streptococcus* (GBS) strains [ATCC 12386 (Ia), ATCC 12403 (III), and ATCC 49446 (IV)] and one non-hemolytic GBS serotype [ATCC 13813 (Ic)] each at two concentrations (~1x and ~3x LoD), as well as two negative samples were tested by two operators in triplicate on six non-consecutive days at three sites for a total of 108 results for each sample (2 operators x 3 replicates/day x 6 days x 3 sites). Three lots of the Xpert GBS LB XC test were used at each of the three sites. Testing was performed on the GeneXpert Dx System GX-XVI (site 1), the GeneXpert Infinity-80 (site 2), and the GeneXpert Dx System GX-IV and GX-XVI (both at site 3).

GBS bacteria were diluted to pre-determined concentrations in a simulated overnight (18-24 hours) enriched LB sample matrix (containing 0.3% Porcine Mucin, 20% whole blood, 1% urine and 1% stool diluted in PBS) to achieve low positive (~1x LoD) and moderate positive (~3x LoD) concentrations. GBS negative samples consisted of only sample matrix. Panels were randomized so that 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. Of the 78 external control samples tested, 98.7% (77/78) gave a valid result on the first test and 1.3% (1/78) gave an invalid result on the first test. Retest of the one external control sample that initially resulted in a non-determinate result gave a valid result. There was 100% agreement of the Xpert GBS LB XC test results with the expected external control results.

When testing the reproducibility panel, the Xpert GBS LB XC assay had initial valid results 97.9% (1057/1080) of the time with 17 ERRORS and 6 NO RESULT outcomes. Repeat testing was conducted per the package insert. Twenty-two of the 23 repeated tests yielded valid results with an overall valid rate of 99.9% (1102/1103).

A summary of the qualitative reproducibility results are summarized in **Table 1**. All of the negative and moderate positive (~3x LoD) panel members yielded the expected results 100% of the time. The percent agreement with the expected result was >95% for all low positive (~1x LoD) panel members, which is acceptable.

Table 1. Percent Agreement[1] of Qualitative Reproducibility Results

Panel Member	LoD	Site 1		Site 2		Site 3		%Total Agreement with 95% CI
		Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
GBS serotype Ia	~1x	100% (18/18))	94.4% (17/18)	100% (18/18)	94.4% (17/18)	100% (18/18)	100% (18/18)	98.1% (106/108) 93.5-99.9
	~3x	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100

Panel Member	LoD	Site 1		Site 2		Site 3		%Total Agreement with 95% CI
		Op 1	Op 2	Op 1	Op 2	Op 1	Op 2	
GBS serotype Ic	~1x	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100
	~3x	100% (17/17)[2]	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (107/107) 96.5-100
GBS serotype III	~1x	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100
	~3x	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100
GBS serotype IV	~1x	100% (18/18)	100% (18/18)	94.4% (17/18)	94.4% (17/18)	100% (18/18)	94.4% (17/18)	97.2% (105/108)
	~3x	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100
Negative 1	-	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100
Negative 2	-	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (108/108) 96.6-100

Op: operator
[1]Percent agreement with expected result.
[2]One replicate excluded due to non-determinate result after repeat testing.

The data was also analyzed to the evaluate the repeatability (within-run variance/assay precision) as well as the within-laboratory precision of the underlying quantitative result (Ct values) obtained from the Xpert GBS LB XC, on which the qualitative reportable results are based (**Table 2**).

Table 2. Summary of Ct Variance (by variance source)

Panel Member	LoD	N	Mean Ct	Site		Operator		Lot		Day		Within-Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
GBS serotype Ia	~1x	106[1]	34.6	0.6	16.0	0	0	0.7	23.2	0	0	1.1	60.8	1.5	4.3
	~3x	108	32.7	0.1	0.6	0	0	0.3	11.0	0	0	0.9	88.4	0.9	2.9
GBS serotype Ic	~1x	108	35.2	0	0	0.2	2.7	0	0	0.6	21.5	1.0	75.8	1.2	3.4
	~3x	107[2]	33.5	0	0	0	0	0.3	11.0	0.5	34.9	0.6	54.1	0.8	2.4
	~1x	108	35.0	0.3	4.0	0	0	0.2	2.6	0.5	14.9	1.1	78.5	1.3	3.6

Panel Member	LoD	N	Mean Ct	Site		Operator		Lot		Day		Within-Run		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
GBS serotype III	~3x	108	33.9	0	0	0.2	5.6	0.3	10.7	0	0	0.9	83.6	0.9	2.8
GBS serotype IV	~1x	105[3]	34.9	0	0	0.5	19.1	0.2	3.1	0.2	3.9	1.1	73.9	1.2	3.6
	~3x	108	33.3	0	0	0.2	4.1	0.4	21.0	0.1	1.0	0.8	73.9	1.0	2.9
Negative 1[4]		108	32.1	0.1	0.7	0	0	0.3	18.2	0.2	10.7	0.6	70.4	0.7	2.2
Negative 2[4]		108	31.9	0	0	0	0	0.4	26.8	0.2	5.6	0.6	67.6	0.7	2.1

N: number of samples analyzed
SD: Standard Deviation
CV: Coefficient of Variation
[1] Two panel members with GBS-negative results were excluded from the analysis.
[2] One sample with a GBS-negative result was excluded from the analysis.
[3] Three panel members with GBS-negative results were excluded from the analysis.
[4] Analysis based on the SPC Ct values for the negative panel members.

2. Linearity:

   Not applicable.

3. Analytical Specificity/Interference:

   Analytical Specificity
   The analytical specificity of the Xpert GBS LB XC test was evauated by testing a panel of 128 strains representing bacterial, viral, parasite and yeast strains commonly found in vaginal and rectal flora or phylogenetically related to GBS (**Table 3**). Bacteria were tested at ≥ 1 x 10^6 CFU/mL, except as noted, and viruses and parasites were tested at ≥ 1 x 10^5 organisms or copies/mL in simulated sample matrix. Microorganisms with potential to grow to high titers in LB during enrichment (i.e., *Candida albicans, Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Streptococcus anginosus, Streptococcus parasanguinis, Corynebacterium accolens*) were tested at > 1x10^8 CFU/mL in LB clinical sample matrix. One hundred and twenty one (121) of 128 strains were tested individually or in pools of 2-6 organisms in the absence or presence of GBS serotype 1a at 3x LoD. The remaining seven strains (*Finegoldia magna, Mobiluncus curtisii subsp. curtisii, Peptoniphilus asaccharolyticus, Fusobacterium nucleatum, Peptostreptococcus anaerobius, Anaerococcus tetradius* and *Anaerococcus prevotii*) were not available for *in vitro* testing and were evaluated by *in silico* analysis using the Xpert GBS LB XC 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.

   No cross-reactivity or interference of GBS detection was observed or predicted.

Table 3. Organisms Evaluated for Analytical Cross-Reactivity and Interference of GBS Detection

Organism			
Abiotrophia defectiva	*Enterobacter cloacae*	*Mycoplasma genitalium*[b]	*Stenotrophomonas maltophilia*
Acinetobacter baumannii	*Enterococcus durans*	*Neisseria gonorrhoeae*	*Streptococcus acidominimus*
Acinetobacter lwoffii	*Enterococcus faecalis*	Norovirus	*Streptococcus anginosus*
Actinobacillus pleuropneumoniae	*Enterococcus faecium*	*Pantoea agglomerans*	*Streptococcus bovis*
Aeromonas hydrophila	*Enterococcus gallinarum*	*Pasteurella aerogenes*	*Streptococcus canis*
Alcaligenes faecalis	Epstein-Barr virus	*Peptoniphilus asaccharolyticus*[a]	*Streptococcus constellatus*
Anaerococcus lactolyticus	*Escherichia coli*	*Peptostreptococcus anaerobius*[a]	*Streptococcus criceti*
Anaerococcus prevotii[a]	*Finegoldia magna*[a]	*Porphyromonas asaccharolytica*	*Streptococcus cristatus*
Anaerococcus tetradius[a]	*Fusobacterium nucleatum*[a]	*Prevotella bivia*	*Streptococcus downei*
Arcanobacterium (Trueperella) pyogenes	*Gardnerella vaginalis*	*Prevotella melaninogenica*	*Streptococcus dysgalactiae subsp. dysgalactiae*
Atopobium (Fannyhessea) vaginae	*Giardia lamblia*[b]	*Prevotella oralis*	*Streptococcus dysgalactiae subsp. equisimilis*
Bacillus cereus	*Haemophilus influenzae*	*Propionibacterium acnes*	*Streptococcus equi subsp. equi*
Bacillus coagulans	*Hafnia alvei*	*Proteus mirabilis*	*Streptococcus gordonii*
Bacteroides fragilis	Hepatitis B virus	*Proteus vulgaris*	*Streptococcus intermedius*
Bifidobacterium adolescentis Reuter	Hepatitis C virus	*Providencia stuartii*[b]	*Streptococcus mitis*
Bifidobacterium brevis	Human immunodeficiency virus	*Pseudomonas aeruginosa*	*Streptococcus mutans*
BK virus	Human Papillomavirus 18[b]	*Pseudomonas fluorescens*	*Streptococcus oralis*
Blastocystis hominis[b]	*Klebsiella (Enterobacter) aerogenes*	*Rhodococcus equi*	*Streptococcus parasanguinis*
Bordetella pertussis	*Klebsiella oxytoca*	Rubella virus	*Streptococcus pneumoniae*
Burkholderia cepacia	*Klebsiella pneumoniae*	*Salmonella enterica subp. typhimurium*	*Streptococcus pseudoporcinus*
Campylobacter jejuni	*Lactobacillus acidophilus*	*Salmonella enterica subsp. enterica ser. Dublin (group D)*	*Streptococcus pyogenes*[b]
Candida albicans	*Lactobacillus casei*	*Serratia liquefaciens*	*Streptococcus ratti*
Candida glabrata	*Lactobacillus delbrueckii lactis*	*Serratia marcescens*	*Streptococcus salivarius*
Candida tropicalis	*Lactobacillus gasseri*	*Shigella flexneri*	*Streptococcus sanguinis*
Chlamydia trachomatis	*Lactobacillus plantarum*	*Shigella sonnei*	*Streptococcus sobrinus*
Citrobacter freundii	*Lactobacillus reuteri*	*Staphylococcus aureus*[c]	*Streptococcus suis*
Clostridium difficile	*Listeria monocytogenes*	*Staphylococcus epidermidis*	*Streptococcus uberis*
Corynebacterium accolens	*Micrococcus luteus*	*Staphylococcus haemolyticus*	*Streptococcus vestibularis*
Corynebacterium sp. (genitalium)	*Mobiluncus curtisii subsp. curtisii*[a]	*Staphylococcus intermedius*	*Toxoplasma gondii*
Corynebacterium urealyticum	*Moraxella atlantae*	*Staphylococcus lugdunensis*	*Trichomonas vaginalis*
Cryptococcus neoformans	*Moraxella catarrhalis*	*Staphylococcus saprophyticus*	*Vibrio cholerae*
Cytomegalovirus	*Morganella morganii*	*Staphylococcus simulans*	*Yersinia enterocolitica*

[a] Evaluated *in silico*
[b] Evaluated with DNA
[c] Tested $<1x10^6$ ($2x10^5$ CFU/mL)

Interference

To evaluate the potential inhibitory effects of endogenous and exogenous substances when using the Xpert GBS LB XC, potentially interfering substances (listed in **Table 4)** were tested at concentrations close to saturation. Eight endogenous and 25 exogenous substances were evaluated in six replicates in the absence and presence of GBS (serotype 1a, ATCC 12386) at ~3x LoD (1989 CFU/mL). 75 µL of each liquid substance (with and without GBS) was added onto the swab by pipetting. Solid substances were added on the swab by dipping ¾ of the swab head into the substance. 75 µL of simulated sample matrix with or without GBS at 3x LoD were then pipetted onto the undipped part of the swab head. Tablets were dissolved in simulated sample matrix with or without GBS at 3x LoD at their highest soluble concentration. 75 µL of each sample was added onto the swab by pipetting.

Five exogenous substances (Aquasonic gel, Floraplus, Pepto Bismol, Skin oil and Xyloproct) were tested at lower concentrations to determine the highest tolerated amount on the swab. There was no interference in the presence of the substances at the concentrations listed in **Table 4**. All positive and negative GBS samples were correctly identified using the Xpert GBS LB XC test.

Table 4. Substances Evaluated for Interference of GBS Detection

Substance (Source)	Concentration on Swab
Human Amniotic Fluid (Lee Biosolutions Inc.)	60% (v/v)
Human Urine (Novakemi)	60% (v/v)
Human Whole Blood - EDTA (BioChemed)	80%(v/v)
Human Whole Blood - Na Citrate (BioChemed)	80% (v/v)
Leukocytes, Buffy coat, $2x10^7$ WBCs/ml (Karolinska University Hospital)	80% (v/v)
Meconium (Lee Biosolutions)	100% (w/v)
Mucus (Sigma-Aldrich/Merck)	30% (w/v)
Human Feces - Pool of 10 donors	100 % (w/v)
Anti-Diarrheal Medication (Pepto Bismol)	40 % (v/v)
Anti-Diarrheal Medication (Dimor Comp [Dimeticone] from Nordic Drugs)	0.03% loperamid + 2% dimetkon (w/v)
Lubricant (RFSU Klick Ultra Glide)	100% (w/v)
Lubricant (Sense Me Aqua Glide)	100% (w/v)
Lubricant (KY-Jelly)	100% (w/v)
Body Oil (ACO Repairing Skin Oil)	100% (w/v)
Dialon Baby (Dialon Baby powder)	100% (w/v)
Deodorant Powder (Vagisil Deodorant Powder)	100% (w/v)
Deodorant Spray (LN Intimate Deo)	60% (v/v)
Deodorant Suppositories (Norforms Feminine Deodorant Suppositories)	28.5% (w/v)
Enema solution (Microlax mikrolavemang – McNeil)	100% (w/v)
Oral Laxative (Inolaxol – Mylan)	25% (w/v)
Oral Laxative (Phillips Milk of Magensia – Bayer)	60% (w/v)
Oral Laxative (Pursennid Ex-Lax – GSK)	0.64% (w/v)
Spermicidal Foam (Caya preventivgel)	100% (v/v)
Stool Softener (Laktulos - Meda	60% (v/v)
Stool Softener (Movicol – Norgine)	11% (w/v)
Topical Hemorrhoid Ointment (Xyloproct Rectal Ointment - Aspen)	10% (w/v)
Topical Hemorrhoid Ointment (Scheriproct rektalsalva / Prednisolone Ointment– Bayer)	100% (v/v)

Substance (Source)	Concentration on Swab
Ultrasound Transmission Gel (Aquasonic Gel – Parker)	25% /w/v)
Vaginal Antifungal Gel (Multi-Gyn Actigel)	100% (v/v)
Vaginal Antifungal Gel (Multi-Gyn Floraplus)	75% (w/v)
Vaginal Anti-itch Cream (Ellen Probiotisk Utvärtes Intim Creme)	100% (v/v)
Vaginal Antifungal Cream (Canesten - Bayer)	100% (v/v)
Vaginal Antifungal Cream (Daktar – McNeil)	100% (v/v)

4. Assay Reportable Range:

   Not applicable.

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

   Specimen Stability
   Vaginal/rectal swab stability prior to enrichment in LB: The stability of vaginal/rectal swab specimens was evaluated at various storage conditions for specimens prior to enrichment (i.e., room temperature (25 ±3°C) up to 24 hours and refrigerated (2-8°C) up to 6 days). Positive and negative samples were prepared with simulated sample matrix. The positive sample consisted of one GBS strain (serotype 1a, ATCC 12386) that was spiked at ~3x LoD into simulated matrix while the negative sample consisted of just the simulated matrix.

   Swabs were loaded with 75µl of sample using a pipette and two swabs were placed into the Cepheid collection device which consists of two swabs and a transport tube that contains a polyurethane foam sponge soaked with a non-nutrient media. The two swabs in each transport tube were incubated at 25 ±3ºC or at 5 ±3°C for 30 hours and 7 days, respectively. At each time point, eight positive swabs and four negative swabs were enriched in 5 ml LB medium (at 35-37°C for 18-24 hours) followed by testing with the Xpert GBS LB XC assay. One reagent lot was used during the study.

   All 8 positive and 4 negative samples yielded correct results after incubation at each condition to support a specimen stability claim for samples prior to enrichment in room temperature (25±3°C) for up to 24 hours or refrigerated (2-8°C) for up to six days. However, since the stability study was performed using simulated sample matrix rather than natural clinical matrix, specimen stability claims are limited to conditions evaluated in the clinical study. As such, the stability claim for samples prior to enrichment is room temperature (25±3°C) for up to 24 hours or refrigerated (2-8°C) for up to two days.

   Enriched culture stability in LB: Swabs were prepared as described above. Swabs were stored in pairs and stored in an incubator at 25 ±3ºC for 27 – 30 hours, in an incubator at 2-8°C for 7 days, and in a refrigerator at 5 ±3°C for 7 days. At the respective time point, eight positive swabs and four negative swabs were enriched in 5 mL (at 35-37°C for 18-24 hours). After performing time 0 testing, LB vials were stored in a refrigerator (5 ±3ºC) for four days followed by testing using the Xpert GBS LB XC assay. One reagent lot was used during the study.

All 8 positive and 4 negative samples yielded correct results after incubation at each condition supporting a specimen stability claim for enriched LB samples refrigerated (2-8°C) for up to 72 hours.

External Controls

One GBS positive external control and one GBS negative external control were tested on each day of clinical study testing. The test results for the positive control was considered valid if they provided a "GBS POSITIVE" result. The valid test result for the negative control was "GBS NEGATIVE". Of the 94 external control samples tested, 92 gave a valid result on the first test (92/94 = 97.9% valid rate). Two external controls initially gave indeterminate results (2/94 = 2.1% indeterminate rate), but gave valid, expected results after retesting. There was 100% agreement for all final external control results with the Xpert GBS LB XC test when compared to the expected results.

6. Detection Limit:

A study was performed to determine the limit of detection (LoD) of the Xpert GBS LB XC test with 12 GBS strains representing 12 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 19/20 replicates were positive.

To support use of analytical studies with a simulated sample matrix, five of the 12 strains (serotypes Ia, Ic, III, IV, V) were first spiked into GBS-negative clinical Lim broth (LB) matrix to estimate the LoD. The most common serotypes (Ia, III, V) were evaluated with two reagent lots and the remaining serotypes (Ic and IV) were evaluated with one reagent lot. Each strain was diluted to seven concentrations (1500 – 5.86 CFU/mL) to generate serotype panels. Each panel was tested in 24 replicates over three days. Data was analyzed with Probit regression analysis to generate a preliminary LoD. In addition, 30 negative replicates (i.e., no GBS spiked) were tested with each serotype panel and reagent lot.

The LoD of all 12 strains were then estimated in a similar manner using simulated sample matrix. For serotypes with LoDs estimated using two reagent lots, the highested observed LoD was used for verification. The preliminary LoDs were verified by spiking each of the 12 GBS serotype strains into simulated sample matrix at the 95% upper confidence level concentration determined in the Probit analysis. Each strain was tested in 20 replicates with a third reagent lot. The LoD of each strain is summarized in **Table 5**.

Table 5. Xpert GBS LB XC LoD of GBS Serotypes

GBS Serotype	Strain ID and Source	CFU/mL (Probit)		Verified LoD
		Clinical Matrix[1]	Simulated Matrix[2]	
Ia	ATCC 12386	375 (682, 230)	1500, 750 (663, 282)	663
Ib	ATCC 12401		93.75 (40)	40
Ic*	ATCC 13813	375(308)	375 (301)	301
II*	ATCC 12973		375 (173)	173
III	ATCC 12403	375 (297, 290)	750, 375 (540, 299)	540
IV	ATCC 49446	187.5, 1500 (538)	750 (429)	429
V	ATCC BAA-23	750, 375 (349, 284)	750, 750 (501, 315)	618[3]

GBS Serotype	Strain ID and Source	CFU/mL (Probit)		Verified LoD
		Clinical Matrix[1]	Simulated Matrix[2]	
VI	CDC ID: 201022816		750 (449)	544[3]
VII	CDC ID: 4832-06		750 (620)	620
VIII	CDC ID: 5030-08		1500 (682)	682
IX	ATCC BAA-2668		750 (465)	465
X	ATCC 49449		750 (677)	677

*Non-hemolytic strains.

[1] Twenty-four of the 1615 tested assay cartridges provided indeterminate test results (ERROR/INVALID/NO RESULT). All 24 samples were retested and generated valid or indeterminate results.

[2] Thirty-three of the 3402 tested assay cartridges provided indeterminate test results (ERROR/INVALID/NO RESULT). Thirty-one of the 33 samples were retested and generated validor indeterminate results.

[3] LoD determined by the 95% CI upper limit due to an 85% Hit rate during verification.

7. Assay Cut-Off:

   The preliminary cut-off for the Xpert GBS LB XC assay was established and validated by evaluating the performance using 1188 prospective specimens (198 positive and 990 negative by the reference culture method). Based on the established cut-off, the Xpert GBS LB XC assay demonstrated an overall sensitivity of 99.0% and specificity of 88.8% when compared to the reference culture method. The cut-off was further validated in an independent clinical study, described below.

8. Accuracy (Instrument):

   Not applicable

9. Carry-Over:

   The Xpert GBS LB XC test uses single-use disposable cartridges that hold the PCR reagents and host the PCR process. To demonstrate that these cartridges prevent specimen and amplicon carry-over contamination, 21 runs alternating between GBS negative and high titer GBS positive samples (GBS serotype 1a spiked at $7.5x10^{-5}$ CFU/swab into simulated sample matrix) were performed consecutively on two GeneXpert modules (for a total of 42 run). All 20 positive samples correctly reported as GBS positive while all 22 negative samples were correctly reported as GBS negative. No evidence of carry-over contamination was observed as all negative samples in the study produced the expected “GBS Negative” result.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable

2. Matrix Comparison:

   To support use of a simulated sample matrix in various analytical studies, a matrix equivalency study was performed to demonstrate comparable Xpert GBS LB XC test

performance with a Lim broth clinical sample matrix and simulated sample matrix. GBS bacteria subtype 1a (ATCC 12386) and serotype III (ATCC 12403) were each spiked into both matrices at three levels (5x LoD, 2x LoD, 0.2x LoD) based on the highest LoD previously determined in LB clinical matrix (**Table 5**). Matrices without GBS were used as negative samples. A summary of the results is provided in **Table 6**. Even though the assay is qualitative, the average Ct difference between the simulated and LB clinical sample matrices at each GBS level was evaluated to further confirm equivalency. The Ct difference of < 1 Ct was observed confirming that the results are comparable. All results met acceptance criteria.

Table 6. Xpert GBS LB XC Matrix Equivalency Study Results

Matrix	GBS Level	Expected % Positive	No. of Replicates	No. Positive (%)	
				GBS Serotype 1a	GBS Serotype III
Simulated Sample Matrix	5x LoD	100	10	10 (100%)	10 (100%)
	2x LoD	≥95	30	30 (100%)	30 (100%)
	0.2x LoD	10-90	20	13 (65%)	7 (35%)
	Negative	0	10	0 (0%)	0 (0%)
LB Clinical Sample Matrix	5x LoD	100	10	10 (100%)	10 (100%)
	2x LoD	≥95	30	30 (100%)	29 (96.7%)
	0.2x LoD	10-90	20	11 (55%)	10 (50%)
	Negative	0	10	0 (0%)	0 (0%)

C Clinical Studies:

1. Clinical Sensitivity:

Performance characteristics of the Xpert GBS LB XC test were evaluated at four institutions in the U.S. using the GeneXpert Dx instrument system. Dual vaginal/rectal swab specimens were collected from pregnant women (≥36 – <37 weeks gestation) for GBS testing as a part of routine care. Specimens were inoculated in Lim broth (LB), per standard of care, and enriched for 18-24 hours. Enriched specimens that met inclusion criteria were enrolled, de-identified and stored at 2-8°C until tested with the Xpert GBS LB XC test, within 72 hours of the completion of LB incubation. An aliquot of the LB enriched specimen was shipped refrigerated with frozen gel packs to the reference laboratory for bacterial culture within 72 hours of the completion of incubation.

The Xpert GBS LB XC performance was compared to a composite comparator, consisting of an FDA-cleared molecular assay for Group B *Streptococcus* detection and a reference culture procedure. The reference culture procedure consisted of incubating the vaginal/rectal swabs for 18-24 hours at 35ºC, subculturing the LB enriched samples on tryptic soy agar with sheep blood (SBAP) and Columbia Naladixic Acid Agar (CNA) plates followed by incubation for 18-24 hours at 35ºC. 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 subcultured on SBAP plates for organism identification by MALDI-TOF. A total of 627 specimens were enrolled in the study; five were excluded from testing due to protocol deviations. Per the package insert, in the event of indeterminate results with the Xpert GBS LB XC test (i.e., INVALID, ERROR, or NO RESULT), a single retest was performed if the remaining LB enriched specimen volume was sufficient. If the repeat test result was also indeterminate, it was reported as such. If the repeat test result generated a

valid result, the valid result was reported. Nine of the 622 specimens tested generated an initial indeterminate result with the Xpert GBS LB XC test (9/622 = 1.4% indeterminate rate). Eight of the nine specimens generated a valid result after retesting the specimen. The result from the remaining indeterminate result was removed from the final analysis for a total of 621 valid Xpert GBS LB XC results. As summarized in **Table 7**, the Xpert GBS LB XC demonstrated an overall sensitivity and specificity for detection GBS of 99.3% and 98.7%, respectively, relative to the composite comparator result.

Table 7. Xpert GBS LB XC performance compared to composite comparator

		Composite Comparator[1]		
		GBS Positive[2]	**GBS Negative[3]**	**Total**
Xpert GBS LB XC	**GBS Positive**	142	6	148
	GBS Negative	1	472	473
	Total	143	479	621
Sensitivity: 99.3% (142/143) [95% CI: 96.1-99.9%] Specificity: 98.7% (472/479) [97.3-99.4%] PPV: 95.9% (142/148) [95% CI: 91.4-98.1%] NPV: 99.8% (472/473) [95% CI: 98.8-100%]				

PPV: Positive Predictive Value
NPV: Negative Predictive Value
[1]Composite comparator consisted of the reference method (enriched culture and MALDI-TOF identification) and an FDA-cleared molecular test.
[2]A positive result for either the reference method or the FDA-cleared molecular assay yielded a final positive comparator result.
[3]A negative result for both the reference method and the FDA-cleared molecular test yielded a final negative comparator result.

2. Clinical Specificity:

   See Section VII.C.1. (Clinical Sensitivity)

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 Xpert GBS LB XC test was evaluated in a four-site prospective clinical study in the U.S. using enriched Lim Broth cultures of vaginal/rectal swabs from antepartum women. Overall, the percent positivity for GBS as determined by the Xpert GBS LB XC test was

23.8% (148/621) and the prevalence of GBS as determined by the composite comparator was 23.0% (143/621).

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

VIII Proposed Labeling:

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

IX Conclusion:

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