Cepheid

Instructions For Use

Xpert® GBS LB XC

For use with GeneXpert® System with Touchscreen

Catalog Numbers

REF GXGBSLBXC-10

REF GXGBSLBXC-120

303-0944 | Rev. B | 2024-05

℞only IVD *In Vitro* Diagnostic Medical Device

Trademark, Patents, and Copyright Statements

See Revision History for a description of changes.

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Getting Started

Product Information

Proprietary Name

Xpert® GBS LB XC

Common or Usual Name

Xpert GBS LB XC

Intended Use, Summary, and Principle of Procedure

Intended 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.

Summary and Explanation

Group B *Streptococcus* (GBS) bacterial infection is associated with rare but serious illness in infants born to women who are colonized with *Streptococcus agalactiae*. Illness can occur in the first 7 days after birth (early-onset disease) or between a week and a few months after birth (late-onset disease). Infants with GBS infection can present with sepsis, pneumonia, or meningitis.[1,2,3,4]

The standard of care for preventing neonatal (early-onset) GBS disease, updated in 2019, indicates screening of pregnant women at 36 0/7 – 37 6/7 weeks of gestation to determine their GBS colonization status.[1]

The CDC published a revised guideline in November 2010 recommending, as an alternative to culture-based testing, that vaginal/rectal specimens could be tested using a nucleic acid amplification test (NAAT) after an 18–24 hour incubation period in an appropriate enrichment broth medium to enhance the detection of GBS for antepartum specimens.[4,5,6] Most antepartum GBS testing is performed by traditional culture-based methods and typically takes two to three days to finalize results.

Principle of the Procedure

The Xpert GBS LB XC test is an automated in vitro diagnostic test for qualitative detection of DNA from Group B Streptococcus (GBS). The test is performed on the Cepheid GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample processing, nucleic acid purification and amplification, and detection of the target sequence in clinical samples using real-time PCR Polymerase Chain Reaction (PCR).

The primers and probes in the Xpert GBS LB XC test are designed to amplify and detect unique sequences in two 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 *S. agalactiae* DNA.

The GeneXpert Instrument Systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the *GeneXpert System with Touchscreen Operator Manual*.

The Xpert GBS LB XC test includes reagents for the detection of DNA from GBS in Lim broth-enriched vaginal/rectal swabs. 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.

The Xpert GBS LB XC has an Early Assay Termination (EAT) function that enables early result reporting. EAT is activated when the pre-determined threshold for a positive test result is reached before the full number of PCR cycles have been completed.

After collecting and transporting a swab specimen 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 sample chamber of the cartridge. The GeneXpert System cartridge is loaded on the GeneXpert Instrument Systems 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 Instrument Systems from measured fluorescent signals and embedded calculation algorithms and are shown in the **View Results** window in tabular and graphic formats. It also reports if the test is invalid, has encountered an error or produces no result.

Reagents, Instruments, and Materials

Reagents

Materials Provided

The Xpert GBS LB XC kit (GXGBSLBXC-10) contains sufficient reagents to process 10 patient or quality-control specimens. The Xpert GBS LB XC kit (GXGBSLBXC-120) contains sufficient reagents to process 120 patient or quality-control specimens. The kits contain the following:

Xpert GBS LB XC Cartridges with Integrated Reaction Tubes	**10 per kit**	**120 per kit**
Bead 1, Bead 2, and Bead 3 (freeze-dried)	3 per cartridge	3 per cartridge
Reagent 1 (Tris buffer with EDTA and surfactants)	3.0 mL per cartridge	3.0 mL per cartridge
Reagent 2 (Sodium Hydroxide)	1.5 mL per cartridge	1.5 mL per cartridge
CD	**1 per kit**	**1 per kit**

- Assay Definition File (ADF)
- Instructions to import ADF into software
- Instructions for Use (Package Insert)

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

Materials Required but not Provided

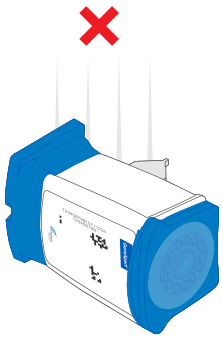
- For GeneXpert system with touchscreen: GeneXpert instrument, touchscreen unit with built-in scanner, Cepheid OS software version 2.0 or higher, and operator manual.
- Lim broth 5mL (Todd Hewitt broth supplemented with 15 µg/mL of nalidixic acid and 10 µg/mL colistin)
- Single use disposable swabs (part number SDPS-120) for processing Lim broth specimens

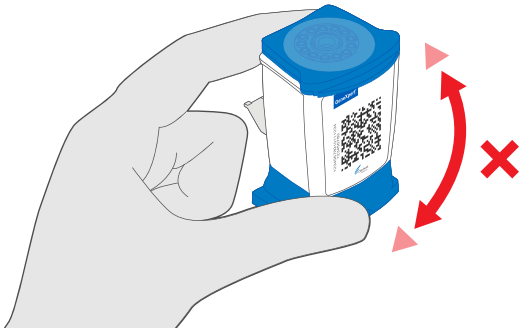
Materials Available but not Provided

- Cepheid Collection Device (part number 900-0370) or equivalent collection device consisting of a collection swab and transport tube with non-nutrient media.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.

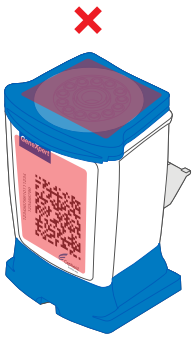
Warnings and Precautions

- For *In Vitro* Diagnostic Use. Rx only
- Treat all biological specimens, including used cartridges and reagents, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute.[8]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert GBS LB XC test does not provide antibiotic susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.
- Do not open the Xpert GBS LB XC cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped after removing it from the packaging.



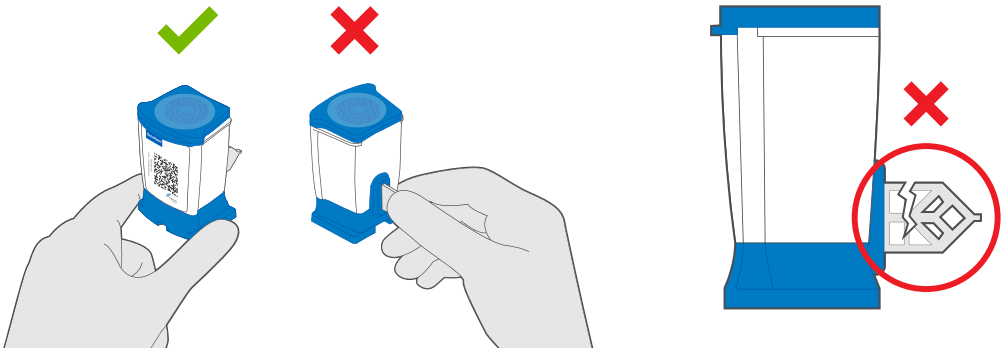


- Do not shake the cartridge. Shaking or dropping the cartridge after opening the lid may yield an erroneous result.
- Do not use a visibly damaged cartridge.
- Do not place the sample ID label on the cartridge lid or on the bar code label.



- Hold the cartridge by the base. Do not touch the reaction tube at the rear of cartridge, as this could cause

damage that would interfere with light passing through it during the test, Do not use a cartridge with a damaged reaction tube.



- Each single-use Xpert GBS LB XC cartridge is used to process one test. Do not reuse cartridges.
- Reagent 2 contains sodium hydroxide (pH > 12.5); (H302, H315, H319) which is irritating to eyes and skin requiring eye and skin protection.
- Clean the work surface/areas with 10% bleach before and after processing Xpert GBS LB XC specimens.
- Specimens can contain high levels of organisms. Ensure that specimen containers do not contact one another. Change gloves if they come in direct contact with the specimen and after the processing of each specimen to avoid contaminating other specimens.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.
- Reliable results are dependent on adequate specimen collection, transport, storage and processing. Incorrect test results may occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the limit of detection of the test. Careful compliance with the instructions for use and the *GeneXpert System with Touchscreen Operator Manual* are necessary to avoid erroneous results.
- GBS stability with the Cepheid Collection Device swab prior to and after enrichment was established analytically with cultured GBS in a simulated specimen matrix. The stability of GBS with other collection devices and transport systems has not been evaluated.

Chemical Hazards, Storage and Handling

Chemical Hazards[9,10]

Reagent 2 (Sodium Hydroxide)

- UN GHS Hazard Pictogram(s):
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Causes skin irritation
  - Causes serious eye irritation
- **Precautionary Statements**

- **Prevention**
  - Wash thoroughly after handling.
  - Wear protective gloves/protective clothing/eye protection/face protection
- **Response**
  - IF ON SKIN: Wash with plenty of soap and water.
  - Take off contaminated clothing and wash before reuse.
  - If skin irritation occurs: Get medical advice/attention.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - If eye irritation persists: Get medical advice/attention.
- **Storage/Disposal**
  - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

Storage and Handling

- Store the Xpert GBS LB XC cartridges and reagents at 2 °C to 28 °C.
- Do not use cartridges that have passed the expiration date on the label.
- Do not use a cartridge that has leaked.
- Do not open the cartridge lid until you are ready to perform testing.

Specimen Collection, Testing, and Results

Specimen Collection

Specimen Collection, Transport and Storage

To obtain adequate specimen, follow the instructions in this section closely.

Using the Cepheid Collection Device or equivalent swab in a non-nutritive transport medium collect a vaginal/rectal swab specimen according to ACOG recommendations.[1] Transport swab specimen to the laboratory for Lim broth enrichment.

The stability of GBS with the Cepheid Collection Device prior to and after enrichment has been evaluated with GBS in a simulated matrix designed to mimic vaginal rectal specimen. For use of specimens collected with an equivalent swab and transport device, refer to the manufacturers information.

- Swab specimens may be stored at 2–8 °C for up to two days before processing in Lim broth for enrichment.
- Swab specimens may be stored at room temperature for up to 24 hours before Lim broth enrichment.

For Lim broth enrichment, follow American Society for Microbiology recommendations for sample enrichment.[5] Place swab in Lim broth and incubate for 18–24 hours at 35–37 °C. The enriched Lim broth is stable at 2–8 °C for up to 72 hours.

Procedure

Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

Note Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

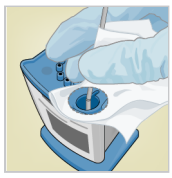
1. Wear protective disposable gloves.
2. Remove the cartridge from the package.

3. Inspect the test cartridge for damage. If damaged, do not use it.
4. Label the cartridge with sample identification.
5. Open the lid of the test cartridge.

6. Prepare the swab as follows:
   a. Mix the Lim broth tube by vigorous shaking or vortexing for 5 seconds.

   b. Dip a clean single use disposable swab (SDPS-120) in the Lim broth.

7. Transfer the swab into the Xpert GBS LB XC cartridge sample chamber as follows.
   a. Raise the swab so that the score mark is centered in the notch.
   b. Break the swab by snapping the shaft to the right.
   Note To minimize contamination, Cepheid recommends using a new lint free wipe or gauze as a shield when breaking the swab into the cartridge chamber.



   c. Ensure the swab is properly positioned in the cartridge and the swab end is not in the notch of the sample chamber opening and does not prevent lid closure.

Correct Swab Placement. Make sure the swab can float freely in the chamber.

Incorrect swab placement. Swab end is caught in the notch of the sample chamber opening.

If the swab is stuck in the notch, use a gloved hand to loosen it from the notch. Use a lint free wipe, gauze or the remaining end of the swab to release it from the notch to minimize the risk of contamination.

8. Close the cartridge lid.

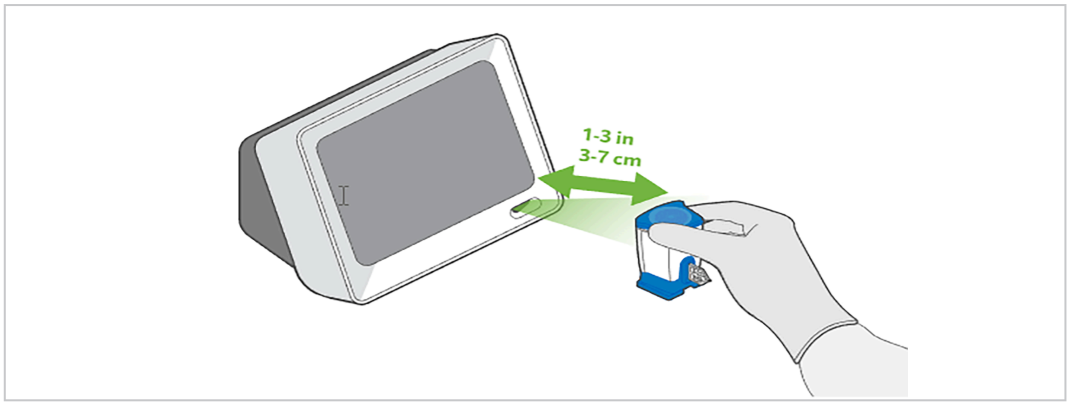
Starting the Test: GeneXpert System with Touchscreen

Important **Before you start the test, make sure that:**

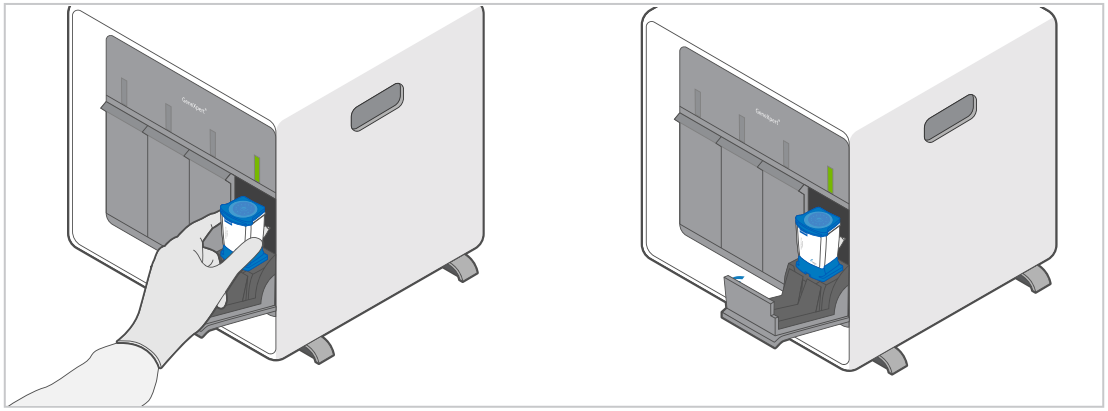
- **The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

Note The default workflow is shown. Your system administrator may alter the workflow.

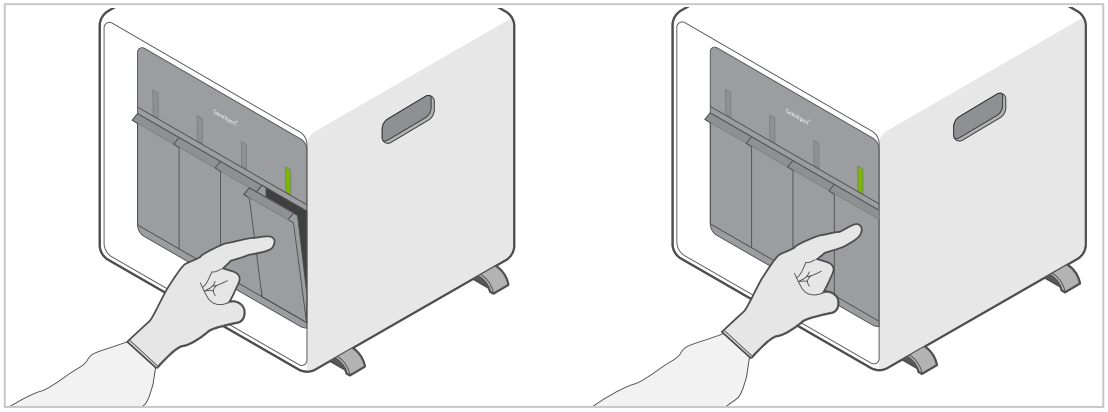
1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, hold the cartridge about 1-3 inches (3-7 cm) away from the scanner. The scanner projects a green crosshair, which you center on the barcode. Scanning is complete when you hear an audible beep. Touch **Continue**.



6. Select the desired test and touch **Continue**.
7. Watch the cartridge preparation video, if needed.
8. On the Confirm screen, review all data and touch **Confirm**.
9. Open the module door under flashing green light and insert the cartridge.



10. Close cartridge module door completely by pressing until it latches. The test starts.



11. When the test completes, the **Results Summary** screen appears. Open the module door and remove cartridge.
12. Dispose of used cartridge in appropriate waste container according to your institution's standard practices.

Viewing Results: GeneXpert System with Touchscreen

The GeneXpert system with touchscreen results screen will automatically interpret test results for you and clearly show them in the **View Results** window.

1. Tap **Results**.
2. Tap the test to be viewed in the Results screen.
3. Click **OK**.
4. To generate a PDF report file, touch **View Report**. More detailed instructions for viewing and uploading results are available in your system operator manual.

Quality Controls

Each test includes a Sample Processing Control (SPC) and a Probe Check Control (PPC).

- **Sample processing control (SPC)**: Ensures the sample was correctly processed. The SPC is included in each cartridge. The SPC monitors the lysis and elution processing. The SPC should pass—generate a valid cycle threshold (Ct) in a negative sample—and may not amplify in a high-positive sample. The SPC passes if it meets the assigned acceptance criteria.

- **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. Probe Check passes if it meets the assigned acceptance criteria.
- **External controls**: may be used in accordance with local, state, and federal accrediting organizations, as applicable.

Results

The results are interpolated by the GeneXpert Instrument Systems from measured fluorescent signals and embedded calculation algorithms and will be shown in the **View Results** window. Possible results are shown in Table 1.

Table 1. GBS Results and Interpretation

Result	Interpretation
`GBS POSITIVE`	GBS target DNA detected • GBS — POSITIVE • SPC — NA (not applicable) • Probe Check Controls—PASS
`GBS NEGATIVE`	GBS target DNA is not detected • GBS — NEG • SPC — PASS • Probe Check Controls—PASS
`INVALID`[a]	Presence or absence of GBS DNA cannot be determined. SPC does not meet acceptance criteria. • GBS — INVALID • SPC — FAIL • Probe Check Controls—PASS
`ERROR`[a]	Presence or absence of GBS DNA cannot be determined. A system component failed, the maximum pressure was reached, or the probe check failed. • GBS — NO RESULT • SPC — NO RESULT • Probe Check Controls—FAIL[b]
`NO RESULTS`[a]	Presence or absence of GBS target DNA cannot be determined. Insufficient data were collected. For example: the operator stopped the test or a power failure occurred during the test. • GBS — NO RESULT • SPC — NO RESULT • Probe Check Controls—NA (not applicable)

a. If an `INVALID`, `ERROR`, or `NO RESULT` occurs, repeat the test according to the instructions in Retest Procedure.

b. If the probe check passed, the error is caused by a system component failure or by exceeding maximum allowable pressure.

Early Assay Termination can reduce the test time for positive results as early as 27 minutes. With GBS negative samples, the test returns results in approximately 43 minutes following the initial 18–24 hour culture enrichment step.

Reasons to Repeat Testing

If any of the test results mentioned below occur, repeat the test according to the instructions in Retest Procedure.

- An **INVALID** result indicates one or more of the following:
  - GBS is not detected and the control SPC failed
  - The sample was not properly processed, or PCR was inhibited
- An **ERROR** result indicates that the test was aborted. Possible causes include: the reaction tube was filled improperly; a reagent probe integrity problem was detected; or the maximum pressure limit was exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.

Retest Procedure

For retest of a **NO RESULT**, **INVALID**, or **ERROR** result, use a new cartridge (do not re-use the cartridge). Use the remaining enriched Lim broth and prepare a new swab as follows. Upon retesting, mix the Lim broth tube by vigorously shaking or vortexing for 5 seconds, then proceed to instruction for testing in Starting the Test: GeneXpert System with Touchscreen

Limitations

Limitations of the Procedure

- 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.
- A negative result does not rule out the possibility of GBS colonization. False negative results may occur if the organism is present at levels below the analytical limit of detection.
- The performance of the Xpert GBS LB XC test was validated using the procedures provided in these instructions for use only. Modifications to these procedures may alter the performance of the test.
- The Xpert GBS LB XC test has been validated with Lim broth medium only. Performance of the test has not been validated with other GBS selective broth enrichment media.
- Culture isolates are needed for performing antibiotic susceptibility testing as recommended for penicillin-allergic women. Use remaining enriched Lim broth to obtain culture isolates. Laboratories must validate their own culture procedures.
- Good laboratory practices should be followed.[8,9]
- Culture test results may be affected by concurrent antibiotic therapy. GBS DNA may continue to be detected following antimicrobial therapy.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- A positive result does not necessarily indicate the presence of viable organisms.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants and may result in a false negative result.

Expected Values

Approximately 10–30% of pregnant women are colonized with GBS in the vagina or rectum.1 GBS colonization can be transient, chronic, or intermittent. Culture screening of both the vagina and rectum for GBS late in gestation during a prenatal care visit can identify women who are likely to be colonized with GBS at the time of delivery.[1,4,5]

During this clinical evaluation for the Xpert GBS LB XC test, prevalence of GBS was 23.0% (143/621) as determined.

Specific Performance Characteristics

Clinical Performance

Performance characteristics of the Xpert GBS LB XC test were evaluated in a multisite study conducted in the United States using the GeneXpert Dx System. Vaginal/rectal swab specimens were collected at three (3) geographically diverse sites from pregnant females for GBS testing as a part of routine care. Specimens were inoculated in Lim broth per institutional policy. For eligible specimens, aliquots of leftover Lim broth samples were obtained for testing with the Xpert GBS LB XC test and patient management continued at the sites per their institutional policies. The Xpert GBS LB XC test was compared to a composite comparator method and an FDA cleared NAAT. The composite comparator method comprises enriched bacterial culture with species identification via MALDI-TOF MS and an FDA cleared NAAT. For the composite comparator, a specimen was considered positive if either enriched bacterial culture or the FDA cleared NAAT was positive and negative when both enriched bacterial culture and the FDA cleared NAAT were negative. Additionally, the Xpert GBS LB XC test was compared directly to the FDA cleared NAAT test.

Results–Performance of Xpert GBS LB XC vs Compositive Comparator

A total of 621 specimens with results from enriched bacterial culture and the FDA cleared NAAT were included in the analyses of Xpert GBS LB XC versus the composite comparator method.

Table 2. Xpert GBS LB XC Performance vs. Composite Comparator

		Composite Comparator		
		Positive	**Negative**	**Total**
Xpert GBS LB XC	**Positive**	142	6	148
	Negative	1	472	473
	Total	143	478	621
Sensitivity: 99.3% (95%CI: 96.1–99.9) Specificity: 98.7% (95%CI: 97.3–99.4) PPV: 95.9% (95%CI: 91.4–98.1) NPV: 99.8% (95%CI: 98.8–100.0) Prevalence: 23.0% (95%CI: 19.9–26.5)				

Sensitivity and specificity of the Xpert GBS LB XC test compared to the composite comparator method were 99.3% and 98.7%, respectively.

Of 622 samples tested with the Xpert GBS LB XC test during this study, 9 yielded non-determinate results on the initial test. These 9 samples were retested and 8 returned valid results. The initial non-determinate rate was 1.4% (9/622) and the final non-determinate rate was 0.2% (1/622).

Analytical Performance

Analytical Sensitivity (Limit of Detection) and Analytical Reactivity (Inclusivity)

The analytical reactivity and limit of detection (LoD) of the Xpert GBS LB XC test were determined for 12 different strains representing 12 known serotypes of GBS, of which 2 were characterized as non-hemolytic (Table 3). Serial dilutions of each serotype were prepared in a Lim broth negative clinical sample matrix or in a simulated sample matrix. Serotypes Ia, III and V were tested with 24 replicates per dilution level for each of two reagent lots across three days. Serotypes Ib, Ic, II, IV and VI-X were tested with one reagent lot for a total of 24 replicates of each dilution level across three days. The LoD was established for each serotype and reagent lot by probit logistic regression analysis.

The LoD for each serotype was verified by testing 20 replicates at the 95% confidence interval upper limit with one reagent lot across three days. The results for all serotypes except serotype V and VI were ≥95% detected (≥19/20) . The result for serotype V and VI was 85% detected (17/20) and the claimed LoD is based on the upper level of 95% confidence interval.

Table 3. GBS Limit of Detection (LoD)

Serotype	LoD (CFU/mL) Probit Results	95% CI Probit Results	Percent Detected	LoD (CFU/mL) Verified	LoD (CFU/swab) Verified
Ia	663	492-835	100%	663	50
Ib	40	32-49	95%	40	3
Ic[a]	301	231-370	100%	301	23
II [a]	173	132-213	100%	173	13
III	540	409-670	100%	540	41
IV	429	324-533	95%	429	32
V	618	384-618	85%	618[b]	46
VI	544	353-544	85%	544[b]	41
VII	620	512-728	100%	620	47
VIII	682	509-855	100%	682	51
IX	465	354-575	100%	465	35
X	677	525-829	95%	677	51

a. Non-hemolytic strain

b. Claimed LoD corresponds to upper 95% upper CI

Analytical Reactivity with GBS *cfb* Mutants

A study was performed to evaluate the analytical reactivity of Xpert GBS LB XC test using GBS strains

containing deletions in or adjacent to the region of the chromosome that encodes the CAMP factor hemolysis gene *cfb*. Ten unique well characterized GBS clinical isolates representing different *cfb* mutations were tested at 833 CFU/mL. All strains with *cfb* mutations were detected with a positivity rate of 100%.

Analytical Specificity (Exclusivity) and Microbial Interference

The analytical specificity of the Xpert GBS LB XC test was evaluated by testing a panel of 128 strains, representing bacterial, viral, parasite and yeast strains commonly found in vaginal/rectal flora or phylogenetically related to GBS (Table 4). Bacteria were tested at ≥ $1x10^6$ CFU/ml, except as noted, and viruses and parasites were tested at a level of ≥ $1x10^5$ organisms, yeast, IU or copies/ml. Microorganisms with potential to grow to high titers in Lim broth during enrichment (*Candida albicans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Streptococcus anginosus*, *Streptococcus parasanguinis*, *Corynebacterium accolens*) were tested at > $1x10^8$ CFU/ml. 121 of 128 strains were tested in Lim broth clinical sample matrix or in simulated sample matrix, both in presence of GBS at 3x LoD and in absence of GBS.

Seven of 128 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, both in silico and in vitro, with any clinically relevant pathogens.

Table 4. Analytical Specificity of Xpert GBS LB XC

Organism		
Arcanobacterium (Trueperella) pyogenes	*Haemophilus influenzae*	*Serratia marcescens*
Atopobium (Fannyhessea) vaginae	*Hafnia alvei*	*Shigella flexneri*
Abiotrophia defectiva	Hepatitis B virus	*Shigella sonnei*
Acinetobacter baumannii	Hepatitis C virus	*Staphylococcus aureus*[a]
Acinetobacter lwoffii	Human immunodeficiency virus	*Staphylococcus epidermidis*
Actinobacillus pleuropneumoniae	Human Papillomavirus 18[b]	*Staphylococcus haemolyticus*
Aeromonas hydrophila	*Klebsiella (Enterobacter) aerogenes*	*Staphylococcus intermedius*
Alcaligenes faecalis	*Klebsiella oxytoca*	*Staphylococcus lugdunensis*
Anaerococcus lactolyticus	*Klebsiella pneumoniae*	*Staphylococcus saprophyticus*
Anaerococcus prevotii[c]	*Lactobacillus acidophilus*	*Staphylococcus simulans*
Anaerococcus tetradius[c]	*Lactobacillus casei*	*Stenotrophomonas maltophilia*
Bacillus cereus	*Lactobacillus delbrueckii lactis*	*Streptococcus acidominimus*
Bacillus coagulans	*Lactobacillus gasseri*	*Streptococcus anginosus*
Bacteroides fragilis	*Lactobacillus plantarum*	*Streptococcus bovis*
Bifidobacterium adolescentis Reuter	*Lactobacillus reuteri*	*Streptococcus canis*
Bifidobacterium brevis	*Listeria monocytogenes*	*Streptococcus constellatus*
BK virus	*Micrococcus luteus*	*Streptococcus criceti*

Organism		
Blastocystis hominis[b]	*Mobiluncus curtisii subsp. Curtisii*[c]	*Streptococcus cristatus*
Bordetella pertussis	*Moraxella atlantae*	*Streptococcus downei*
Burkholderia cepacia	*Moraxella catarrhalis*	*Streptococcus dysgalactiae subsp. dysgalactiae*
Campylobacter jejuni	*Morganella morganii*	*Streptococcus dysgalactiae subsp. equisimilis*
Candida albicans	*Mycoplasma genitalium*[b]	*Streptococcus equi subsp. equi*
Candida glabrata	*Neisseria gonorrhoeae*	*Streptococcus gordonii*
Candida tropicalis	Norovirus	*Streptococcus intermedius*
Chlamydia trachomatis	*Pantoea agglomerans*	*Streptococcus mitis*
Citrobacter freundii	*Pasteurella aerogenes*	*Streptococcus mutans*
Clostridium difficile	*Peptoniphilus asaccharolyticus*[c]	*Streptococcus oralis*
Cytomegalovirus	*Peptostreptococcus anaerobius*[c]	*Streptococcus parasanguinis*
Corynebacterium accolens	*Porphyromonas asaccharolytica*	*Streptococcus pneumoniae*
Corynebacterium sp. (genitalium)	*Prevotella bivia*	*Streptococcus pseudoporcinus*
Corynebacterium urealyticum	*Prevotella melaninogenica*	*Streptococcus pyogenes*[b]
Cryptococcus neoformans	*Prevotella oralis*	*Streptococcus ratti*
Enterobacter cloacae	*Propionibacterium acnes*	*Streptococcus salivarius*
Enterococcus durans	*Proteus mirabilis*	*Streptococcus sanguinis*
Enterococcus faecalis	*Proteus vulgaris*	*Streptococcus sobrinus*
Enterococcus faecium	*Providencia stuartii*[b]	*Streptococcus suis*
Enterococcus gallinarum	*Pseudomonas aeruginosa*	*Streptococcus uberis*
Epstein-Barr virus	*Pseudomonas fluorescens*	*Streptococcus vestibularis*
Escherichia coli	*Rhodococcus equi*	*Toxoplasma gondii*
Finegoldia magna[c]	Rubella virus	*Trichomonas vaginalis*
Fusobacterium nucleatum[c]	*Salmonella enterica subsp. enterica ser. Dublin (group D)*	*Vibrio cholerae*
Gardnerella vaginalis	*Salmonella enterica subp. typhimurium*	*Yersinia enterocolitica*
Giardia lamblia[b]	*Serratia liquefaciens*	

a. Tested < $1x10^6$ ($2x10^5$ CFU/ml)

b. Evaluated with DNA

c. Evaluated *in silico*

Potentially Interfering Substances Study

Substances that may be present in vaginal/rectal specimens with the potential to interfere with the Xpert GBS LB XC test were evaluated. Potentially interfering endogenous and exogenous substances include human amniotic fluid, meconium, urine, fecal material, human blood, lubricating gel, vaginal anti-itch medications, vaginal antifungal medications, anti-diarrheal medications, laxatives, stool softeners, topical hemorrhoid ointments, body oil, body powder, deodorant sprays, enema solutions, and spermicidal foam.

These substances are listed in Table 5. All liquid substances were tested by adding 100% of the substance to the swab, solid substances by covering swab head to 75% and tablets were dissolved to their highest soluble

concentration in simulate sample matrix and added to the swab. Five exogenous substances (Aquasonic® gel, Floraplus, Pepto Bismol®, Skin oil and Xyloproct) were tested at lower concentration to determine the highest tolerated amount on swab (Table 5). The interferents were tested on each swab in the presence and absence of GBS at 3x LoD. There was no interference in the presence of the substances at the concentrations tested in this study. All positive and negative samples were correctly identified using the Xpert GBS LB XC test.

Table 5. Potentially Interfering Substances Tested

Substance	Highest Concentration on Swab Resulting in No Interference
Human Amniotic Fluid	60% (v/v)
Human Urine	60% (v/v)
Human Whole Blood – EDTA	80% (v/v)
Human Whole Blood – Na Citrate	80% (v/v)
Leukocytes, Buffy coat, $2x10^7$ WBCs/mL	80% (v/v)
Meconium	100%
Mucus – mucin from porcine stomach	30% (w/v)
Human Feces – Pool of 10 donors	100%
Anti-Diarrheal Medication – Pepto Bismol	40% (v/v)
Anti-Diarrheal Medication – Dimor Comp [Dimeticone]	0.03% loperamid + 1.7% dimetikon (w/v)
Lubricant – RFSU Klick Ultra Glide	100%
Lubricant – Sense Me Aqua Glide	100%
Lubricant – KY-Jelly	100%
Body Oil – ACO Repairing Skin Oil	100%
Dialon Baby – Dialon Baby Powder	100%
Deodorant Powder – Vagisil® Deodorant Powder	100%
Deodorant Spray – LN Intimate Deo	60% (v/v)
Deodorant Suppositories – Norforms Feminine Deodorant Suppositories	46.4% (w/v)
Enemasolution – Microlax mikrolavemang	100%
Oral Laxative – Mylan	25% (w/v)
OralLaxative – Phillips Milk of Magnesia	60% (v/v)
Oral Laxative – Pursennid Ex-Lax	0.64% (w/v)
Spermicidal Foam – Caya preventivgel	100%
Stool Softener – Laktulos Meda	60% (v/v)
Stool Softener – Movicol	9% (w/v)
Topical Hemorrhoid Ointment – Xyloproct Rectal Ointment	8% (v/v)
Topical Hemorrhoid Ointment – Scheriproct rektalsalva / Prednisolone Ointment	100%
Ultrasound Transmission Gel – Aquasonic Gel	20% (v/v)
Vaginal Antifungal Gel – Multi-Gyn Actigel	100%
Vaginal Antifungal Gel – Multi-Gyn Floraplus	75% (w/v)
Vaginal Anti-itch Cream – Ellen Probiotisk Utvärtes Intim Creme	100%

Substance	Highest Concentration on Swab Resulting in No Interference
Vaginal Antifungal Cream – Canesten	100%
Vaginal Antifungal Cream – Daktar	100%

Carry-over Contamination Study

A study was conducted to demonstrate that no carry-over contamination occurs when testing these single-use, self-contained GeneXpert cartridges in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately following a high GBS positive sample. Twenty-one runs alternating high titer GBS positive and GBS negative samples were performed consecutively on two GeneXpert modules, thus a total of 42 runs were executed for the study. All 20 positive samples were correctly reported as GBS positive. All 22 negative samples were correctly reported as GBS negative.

Reproducibility and Precision

A panel of ten samples with varying concentrations of four different GBS strains were tested by two operators each in triplicate on six different days at three sites (7 samples × 2 operators × 3 times/day × 6 days × 3 sites). Three lots of Xpert GBS LB XC were used at each of the three testing sites. Three strains (serotype Ia, III, IV) represented a hemolytic phenotype and one strain (serotype Ic) represented a non-hemolytic phenotype. The three levels were ~3 × LOD and ~1 × LOD and negative.

Xpert GBS LB XC testing was performed on the GeneXpert Instrument Systems according to the Xpert GBS LB XC test procedure. The percent agreement of the qualitative results for GBS detection for each sample analyzed by each of the six operators and by each site is shown in Table 6. In addition, the overall percent agreement for each sample (total agreement) and the 95% two-sided Wilson Score confidence interval are shown in the last column.

Table 6. Summary of Reproducibility and Precision Results

	Sample	Site 1			Site 2			Site 3			Total Agreement by Sample with 95% CI
		OP 1	OP 2	Subtotal	OP 1	OP 2	Subtotal	OP 1	OP 2	Subtotal	
01	Negative 1[a]	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (108/108) 96.6-100.0
02	Negative 2	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (108/108) 96.6-100.0
03	GBS serotype Ia~1xLoD	100.0% (18/18)	94.0% (17/18)	97.0% (35/36)	100.0% (18/18)	94.0% (17/18)	97.0% (35/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	98.1% (106/108) 93.5-99.8
04	GBS serotype Ic[b]~1xLoD	100.0% (18/18)	94.0% (17/18)	97.0% (35/36)	100.0% (18/18)	94.0% (17/18)	97.0% (35/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.9% (108/108) 96.6-100.0
05	GBS serotype III~1xLoD	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (108/108) 96.6-100.0
06	GBS serotype IV ~1xLoD	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	94.0% (17/18)	94.0% (17/18)	94.0% (34/36)	100.0% (18/18)	94.0% (17/18)	97.0% (35/36)	97.2% (105/108) 92.1-99.4
07	GBS serotype Ia ~3xLoD	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (108/108) 96.6-100.0
08	GBS serotype Ic[b] ~3xLoD	100.0% (17/17)	100.0% (18/18)	100.0% (35/35)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (107/107) 96.5-100.0

	Sample	Site 1			Site 2			Site 3			Total Agreement by Sample with 95% CI
		OP 1	OP 2	Subtotal	OP 1	OP 2	Subtotal	OP 1	OP 2	Subtotal	
09	GBS serotype III ~3xLoD	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (108/108) 96.6-100.0
10	GBS serotype IV ~3xLoD	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (18/18)	100.0% (18/18)	100.0% (36/36)	100.0% (108/108) 96.6-100.0

a. Testing with Serotype Ic was performed separately from testing by the other panel members. Negative panel members were included in both rounds of testing and are represented separately in the performance table above.

b. Serotype Ic is characterized as non-hemolytic.

No statistically significant (p-value of <0.01) differences in performance were between the study sites, the operators, or the cartridge lots used. P-values ranged from 0.7715 to 1.

Appendix

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Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	Consult instructions for use

Symbol	Meaning
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
	Expiration date
	Temperature limitation
	Biological risks
	Caution
	Warning
	For prescription use only

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Revision History

Description of Changes: 303-0944 Rev A to B

Purpose: Corrected two sections

Section	Description of Change
Intended Use	Updated statement.
Analytical Specificity (Exclusivity) and Microbial Interference	Corrected footnote cross references.