Cepheid

Instructions for Use

Xpert® GBS

For use with GeneXpert® System with Touchscreen

Catalog Numbers

REF GXGBS-100N-10

302-9611 | Rev. A | 2023-07

℞only IVD *In Vitro* Diagnostic Medical Device

Trademark, Patents and Copyright Statements

See Revision History for a description of changes.

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Product Information

Proprietary Name

Xpert® GBS

Common or Usual Name

Xpert GBS

Intended Use, Summary, and Principle of Procedure

Intended Use

The Cepheid Xpert GBS test performed on the GeneXpert® Instrument Systems 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. Xpert GBS testing is indicated for rapid identification of antepartum and intrapartum GBS colonization.

- The use of the Xpert GBS test 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 test does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

Summary and Explanation

GBS bacterial infection is associated with serious illness in newborns born to women who are colonized with the microorganism. A recent review estimated that 18% of pregnant women worldwide are colonized with GBS[1] and pregnancy has been associated with a high incidence of invasive GBS disease worldwide with a neonatal attack rate of GBS infection through vertical transmission ranging from 1-2/1000 live births.[2] Transmission of GBS occurs from GBS-colonized women to their newborn before birth (antepartum) or

during birth (intrapartum). In the United States, GBS infection is the major cause of death in newborns who develop sepsis, pneumonia, or meningitis.[3,4,5]

Currently, the standard of care for preventing neonatal GBS disease is screening pregnant women at 36–37 weeks of gestation to determine their GBS colonization status.[4] Most antepartum GBS testing is performed by culture and typically takes two to three days to finalize results. This timing might be adequate for obtaining antepartum GBS culture results for the majority of women; however, some women may not have GBS results available at the onset of labor. The Xpert GBS developed by Cepheid for detecting GBS directly from vaginal/rectal swab specimens takes about 50 minutes or less after testing is initiated.

When GBS status is unknown at the time of labor, the risk-based approach is less effective in identifying colonized mothers than antepartum screening, and susceptibility testing for penicillin allergic women is not possible. For women who have had no prenatal care, or who might deliver preterm, or whose GBS test results are unknown at the time of delivery, intrapartum testing can provide results in time to administer antibiotics before delivery, if required. Xpert GBS testing can be done 24 hours a day, 7 days a week and can be conveniently performed. The potential impact to intrapartum testing is decreased use of unnecessary antibiotics in women not otherwise indicated for prophylaxis, while providing adequate treatment of GBS-colonized women with the resulting decreased risk of neonatal sepsis or meningitis.[7] Effective intrapartum GBS testing for pregnant women who come to labor and delivery without a known GBS status requires prompt specimen collection and capability of providing results quickly enough to initiate recommended duration of antibiotic prophylaxis prior to delivery.

Principle of the Procedure

The GeneXpert Instrument System automates and integrates sample lysis, nucleic acid purification and amplification, and detection of the target sequence in complex samples using real-time and reverse transcription Polymerase Chain Reaction (RT-PCR). The system consists of an instrument, personal computer, and preloaded software for running tests on collected samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination concerns are minimized. For a full description of the system, see the relevant system operator manual.

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

After collecting and transporting a swab sample to the GeneXpert testing area, the swab is inserted into the Xpert GBS cartridge. The GeneXpert Instrument System performs sample preparation by eluting the specimen material from the swab, mixing the sample reagent with the SPC and treatment reagent, capturing cellular material on a filter, lysing the cells, and eluting the DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the integrated reaction tube for real-time PCR and detection. The results are interpolated by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms. Results may be viewed and may be printed. The test process takes approximately 50 minutes or less.

Reagents, Instruments, and Materials

Reagents

Materials Provided

The Xpert GBS kit (GXGBS-100N-10) contains sufficient reagents to process 10 patient or quality-control specimens.

The kit contains the following:

Xpert GBS Test Cartridges with integrated reaction tubes	**10 per kit**
Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
Reagent 1	3.0 mL per cartridge
Reagent 2 (Sodium Hydroxide)	3.0 mL per cartridge
CD • Assay Definition file (ADF) • Instructions to import ADF into GX software • Instructions for Use (Package Insert)	**1 per kit**

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

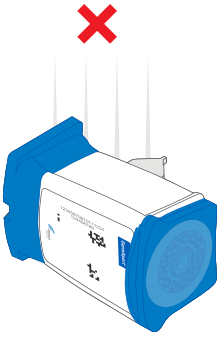
- GeneXpert system with touchscreen: GeneXpert instrument, touchscreen unit with built-in scanner, Cepheid OS software version 2.0 or higher, and *GeneXpert System with Touchscreen Operator Manual*.
- Printer: If a printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Cepheid Collection Device (part number 900-0370)
- Disposable, sterile transfer pipette (for retest only)

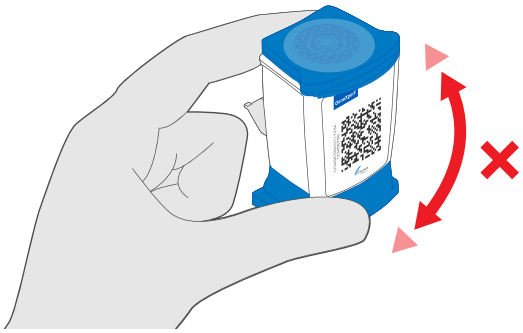
Materials Available but Not Provided

- KWIK-STIK™ (MicroBioLogics, cat. no. 8164: one each of Streptococcus species (Group B) low-level positive control, moderate-level positive control, high-level positive control and L. acidophilus as a negative control)

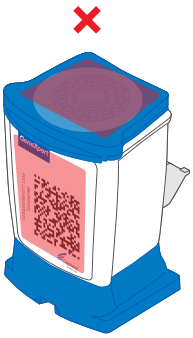
Warnings and Precautions

- For *in vitro* Diagnostic Use. Rx only
- Treat all biological specimens, including used cartridges, 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 universal precautions. Guidelines for specimen handling are available from the U.S. Center for Disease Control and Prevention[8] and the Clinical and Laboratory Standards Institute.[9]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert GBS test does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.
- Do not open the Xpert GBS cartridge lid except when adding the sample.
- Do not load a cartridge that has been dropped or shaken after you have added the sample.

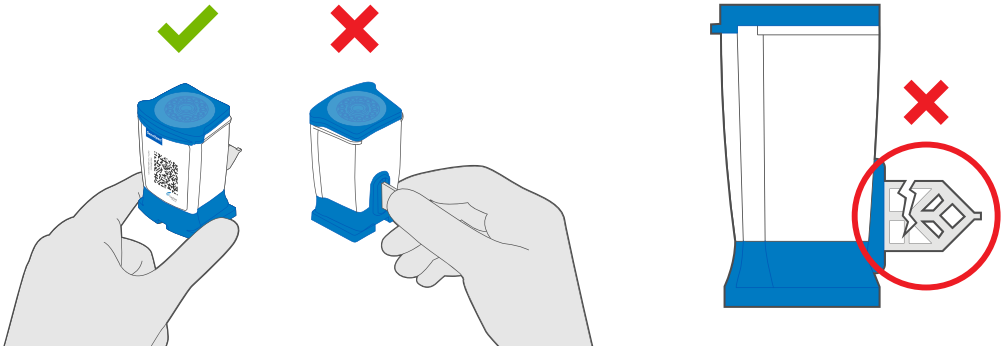




- Do not place the sample ID label on the cartridge lid or on the bar code label.



- Do not use a cartridge that has a damaged reaction tube.



- Do not open used cartridges except for retest and then only to remove eluted sample from the sample chamber with a pipet.
- Each single-use Xpert GBS cartridge is used to process one test. Do not reuse spent cartridges.
- 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.
- Store the Xpert GBS kit at 2–28° C

Chemical Hazards, Storage and Handling

Chemical Hazards[12,13]

- Contains Sodium Hydroxide
- UN GHS Hazard Pictogram:
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Causes skin irritation
  - Causes serious eye irritation
- **Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
    - Do not eat, drink, or smoke when using this product.
    - Avoid release to the environment.
    - 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.
    - Specific treatment, see supplemental first aid information.
    - 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
    - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician if you feel unwell.
    - Rinse mouth.
  - **Storage/Disposal**
    - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations.

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

Storage and Handling

- Store the Xpert GBS kit at 2-28° C.
- Do not use reagents or cartridges that have passed the expiration date.

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, collect specimens according to CDC recommendations.[2] The following procedure should be used:

1. Wipe away excessive amounts of secretion or discharge.
2. Remove both marked swabs from the transport container.
3. Carefully insert both marked swabs into the patient's vagina. Sample secretions from the mucosa of the lower one-third part of the vagina. Rotate the swabs three times to ensure uniform sample on both swabs.
4. Using the same marked swabs, carefully insert both swabs approximately 2.5 cm beyond the anal sphincter, and gently rotate to sample anal crypts.
5. Place both marked swabs in the transport container.
6. If the specimens will be processed within 24 hours, store at room temperature. If the specimens will be tested after 24 hours, refrigerate until testing is performed. Specimens stored at 2–8° C are stable for up to six days.

Procedure

Preparing the Cartridge

Important **Start the test within 15 minutes of adding the sample to the cartridge.**

Note Only one swab is required. The second swab is extra and can be used for susceptibility testing. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women. Do not add 2 swabs to any one cartridge

To add the sample into the cartridge (Xpert GBS):

1. Remove the cartridge from the package.
2. Open the cartridge lid.

3. Remove the marked swabs from the container. Gently brush the two swabs together using a twirling motion so that equal amount of sample is on each swab.
4. Insert one of the swabs into the Xpert GBS sample chamber.
   - Do not insert both swabs into the cartridge.
   - Return the second swab into the collection/transport tube for subsequent antimicrobial susceptibility testing by the microbiology laboratory for GBS positive patients. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.
5. Raise the swab so that the score mark is centered in the notch.
6. Break the swab by snapping the shaft to the right.

7. Close the cartridge lid.

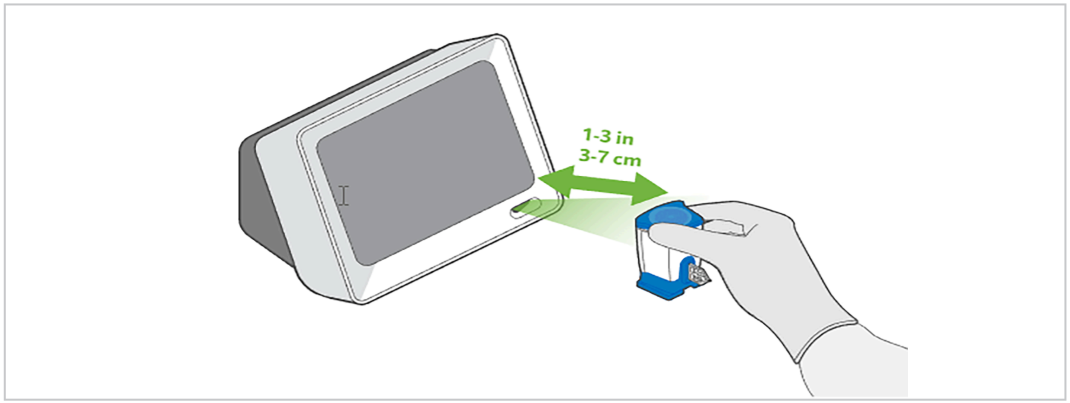
Starting the Test: GeneXpert System with Touchscreen

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

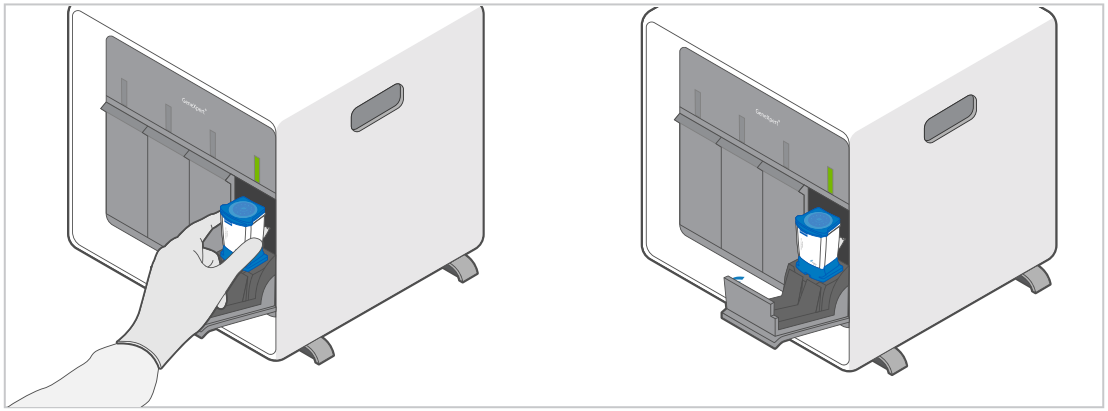
- **The system is running the correct Cepheid OS software version shown in section - Materials Required but Not Provided.**
- **The correct assay definition file is imported into the software.**

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

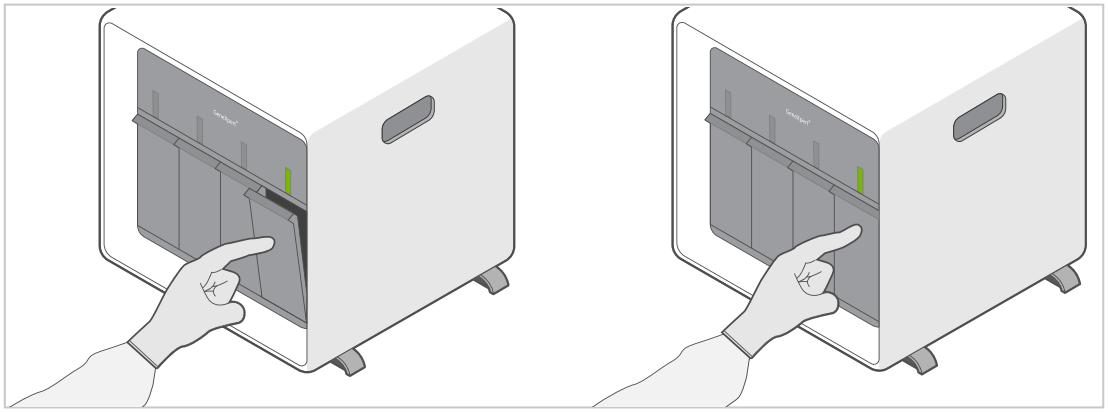
1. Turn on GeneXpert system with touchscreen.
2. Log on to system software using your username and password.
3. On the Modules tab, touch **Start Test**.
4. Follow onscreen prompts to create new test and enter patient and sample information.
5. Scan or manually input the cartridge serial number. If scanning, 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 Control

Each test includes a Sample Processing Control (SPC), and Internal Control (IC) and Probe Check Control

(PCC).

- **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.
- **Internal control (IC)**—Verifies functional PCR reagents and the absence of inhibition that would prevent PCR amplification. The IC should pass—generate a valid Ct in a negative sample—and may not amplify in a high-positive sample. The IC 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**—KWIK-STIK™ (MicroBioLogics, catalog no. 8164) may be used for training, proficiency testing and external QC of the GeneXpert Instrument System. External controls may be used in accordance with local, state, federal accrediting organizations, as applicable. Follow the MicroBioLogics external control procedure described below.

1. Tear open pouch at notch and remove the KWIK-STIK.
2. Pinch the bottom of the ampoule in the cap to release the hydrating fluid.
3. Hold vertically and tap to facilitate flow of fluid through shaft into bottom of unit containing pellet.
4. To facilitate dissolution of the lyophilized cell pellet, crush the pellet and gently pinch the bottom chamber.
5. Pull apart the KWIK-STIK to release the swab, and place the swab in the Xpert GBS cartridge sample chamber.
6. The KWIK-STIK swab is now ready for Xpert GBS testing.

The low-level positive control approximates 620 CFU/swab (~36 Ct) of GBS, and the negative control approximates 17,000 CFU/swab of *Lactobacillus acidophilus* (0 Ct or >42 Ct).

Results

The results are interpolated by the GeneXpert Instrument System 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
POSITIVE	GBS target nucleic acid is detected—presumptive for GBS colonization • SPC—NA (not applicable) • IC—NA (not applicable) • Probe check—PASS The patient's matching second paired swab in the collection/transport tube may be used for antimicrobial susceptibility testing. If necessary the used cartridge can be sent to the microbiology laboratory for back-up susceptibility testing.
NEGATIVE	GBS target nucleic acid is not detected—presumed not colonized for GBS. • GBS—NEG • SPC—PASS • IC—PASS • Probe Check—PASS

Result	Interpretation
INVALID	Presence or absence of GBS cannot be determined. IC and/or SPC does not meet acceptance criteria, or air bubbles formed in the reaction tube. • GBS—INVALID • SPC—FAIL* • IC—FAIL* • Probe Check—PASS * The SPC and/or the IC failed.
ERROR	Presence or absence of GBS cannot be determined. A system component failed, the maximum pressure was reached, or the probe check failed. • GBS—NO RESULT • SPC—NO RESULT • IC—NO RESULT • Probe Check—FAIL* * If the probe check passed, the error is caused by a system component failure.
NO RESULT	Presence or absence of GBS cannot be determined. The operator stopped the test, a power failure occurred during the test, or problems were detected in the cartridge. • GBS—NO RESULT • SPC—NO RESULT • IC—NO RESULT Probe Check—NA (not applicable)

Note If you obtain an **INVALID**, **ERROR**, or **NO RESULT**, testing may be repeated or alternate methods initiated.

- In the event of an **ERROR** result (maximum pressure abort or probe check failure), immediately perform retest or run second swab, or initiate alternate methods. **ERROR** results may happen within first 30 minutes of the test.
- When performing intrapartum testing, repeat testing may not be feasible and will depend on practices and policies within each facility. Coordination between clinicians and the testing laboratory is important to not delay administration of antibiotics while results are pending.

Reasons to Repeat the Test

Repeat the test or initiate alternate procedures if one of the following test results occurs:

- **ERROR**—The test was aborted because a system component failed, the maximum pressure was reached, or the probe check failed.
- **INVALID**—The SPC and/or the IC failed when GBS is negative. An invalid result can also be caused by air bubbles in the reaction tube.
- **NO RESULT**—The operator stopped the test, a power failure occurred during the test, or problems were detected in the cartridge.

If there is fluid in the cartridge sample chamber, use a transfer pipette to transfer all the fluid to the sample chamber of a new cartridge, and then rerun the test. If there is no fluid, use sterile tweezers to transfer the swab to a new cartridge, and then rerun the test. Alternatively, prepare a new cartridge using the second swab, and then rerun the test.

Limitations

Limitations of the Procedure

- The performance of Xpert GBS test was established with the Cepheid GeneXpert Dx System, with vaginal-rectal specimens from antepartum and intrapartum patients collected with the Cepheid Collection Device (part number 900-0370). The use of specimen collection and transport system other than those listed in Materials Required but Not Provided section is not recommended. The use of the Xpert GBS test from other clinical sources has not been assessed and performance characteristics of this test are unknown for other specimen types.
- Erroneous test results might occur from improper specimen collection, technical error, sample mix-up, or because the number of organisms in the specimen is not detected by the test. Careful compliance to the instructions in this insert and to the *Vaginal/Rectal Specimen Collection Protocol* instructions document is necessary to avoid erroneous results. Swabbing both the lower vagina and rectum increases the yield substantially compared with sampling the cervix or sampling the vagina without also swabbing the rectum.
- Because detection of Group B Streptococcus is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage. Testing at 35–37 weeks gestation is recommended to improve sensitivity and specificity of detection of women who remain colonized at the time of delivery.
- Training for personnel using the GeneXpert Instrument System is important to assure accurate and timely results.
- Rerunning the Xpert GBS test when results are **INVALID**, **ERROR**, and **NO RESULT** should depend on practices and policies within each facility. Alternate procedures (e.g., culturing as recommended with selective enrichment broth for 18-24 h) should be available. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women. For culturing, remaining swab specimens should be placed in appropriate transport systems and cultured within 4 days. Culturing from Xpert GBS Reagent 1 has not been validated. Laboratories must validate their own culturing procedures or use the second swab collected to perform culture-based identification and susceptibility testing.
- A positive test result does not necessarily indicate the presence of viable organism. It is, however, presumptive for the presence of Group B Streptococcus.
- Intrapartum testing with Xpert GBS test should be used as an adjunct to other methods available and not used to replace antepartum testing (at 35–37 weeks gestation). The test is not intended to differentiate carriers of Group B Streptococcus from those with streptococcal infection.
- Test results might also be affected by concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.
- Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens or reagents.
- 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.
- A negative result does not rule out the possibility of GBS colonization. False negative results may occur when the GBS concentration in the specimen is below the limit of detection (LOD).

Expected Values

Approximately 10–30% of pregnant women are colonized with GBS in the vagina or rectum. GBS colonization can be transient, chronic, or intermittent. Culture screening of both the vagina and rectum for GBS late in gestation during prenatal care can detect women who are likely to be colonized with GBS at the time of delivery.[4,5] In various studies, sensitivities of 87% (83–92% CI) and 69% (57–79% CI) and specificities of 96% (95–98% CI) and 92% (89–94% CI) have been reported for late-prenatal cultures for identifying colonization status at delivery.[10] During clinical evaluations for the Xpert GBS test, 25.4% (201 of 791) women were colonized with GBS by culture methods.

Specific Performance Characteristics

Clinical Performance

Performance characteristics of the Xpert GBS test were determined in a multi-site prospective investigational study 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 (near-patient test setting). 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 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 test on the GeneXpert Dx System; the other was used in the 2nd NAAT test. The 2nd NAAT test 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[4]: microbiological culture in selective broth medium (LIM broth, which is Todd-Hewitt broth supplemented with 15 µg/ml of nalidixic acid, and 10 µg/ml of colistin), followed by 18–24 h incubation, and subculture onto BAP. Specific identification of colonies suggestive of GBS was done with slide agglutination tests.

The performance characteristics of the Xpert GBS test were determined from the evaluation of laboratory testing done on 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 culture results 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 test 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 test 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%).

Table 2 shows Xpert GBS testing based on the positive and negative GBS culture findings for 791 subjects (3 subjects had cultures that were overgrown or could not be otherwise interpreted). The sensitivity, specificity, and negative and positive value estimates shown are based on results after repeat testing. After repeat testing, 10 cases remained unresolved (n = 781).

Table 2. Xpert GBS Results and Estimated Performance by Patient Category

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

a. All Xpert GBS results are shown after repeat testing. Initial test results are shown in parenthesis.

b. Positive predictive value.

c. Negative predictive value.

d. '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.

e. Three intrapartum women with no results by culture are excluded from the analyses.

f. Overall prevalence of GBS colonization as determined by culture is 25.3%.

g. The subset of intrapartum women who had specimens collected after membrane rupture (rupture of membrane, ROM).

h. The subset of intrapartum women who had specimens collected before membrane rupture (ROM). There would be no biological differences expected between these intrapartum specimens and those collected antepartum.

Table 3. Performance of Xpert GBS and 2nd NAAT Test[A] Relative to Culture

	Xpert GBS						2nd NAAT[a]					
Category	**Sensitivity**	**Lower CI**	**Upper CI**	**Specificity**	**Lower CI**	**Upper CI**	**Sensitivity**	**Lower CI**	**Upper CI**	**Specificity**	**Lower CI**	**Upper CI**
Overall	88.64% (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%
Ante-partum	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%
Intra-partum	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[b]	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[c]	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%

a. The 2nd NAAT targets a sequence in the cfb gene and was previously FDA-cleared.

b. Subset of intrapartum women who had specimens collected after membrane rupture (rupture of membrane, ROM).

c. Subset of intrapartum women who had specimens collected before membrane rupture (ROM).

The following table shows a direct comparison of the 2 PCR tests, Xpert GBS and 2nd NAAT:

Table 4. Direct Comparison of the Two PCR Tests

	Culture Pos			Culture Neg			Culture ND			Total Xpert GBS
	2nd NAAT Pos	2nd NAAT Neg	Unresolved	2nd NAAT Pos	2nd NAAT Neg	Unresolved	2nd NAAT Pos	2nd NAAT Neg	Unresolved	
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	155	44	2	22	567	1	0	3	0	794

Table 5 shows the number of patients tested at each of the six clinical laboratory sites participating in the evaluation, and the estimated performance for Xpert GBS testing (compared to culture findings).

Table 5. Site to Site Comparison

Clinical Test Site	Ante-partum Patients	Intra-partum Patients	Total Patients	Culture Positive	Prev-alence	Xpert GBS with No Results		Sensitivity			Specificity		
						Ini-tially	Re-peating	Estimate	Lower 95%	Upper 95%	Estimate	Lower 95%	Upper 95%
1	0	51	51	11	21.6%	4	1	72.7%	39.0%	94.0%	92.3%	79.1%	98.4%
2	113	5	118	31	26.3%	12	2	90.3%	74.2%	98.0%	97.6%	91.8%	99.7%
3	94	0	94	17	18.1%	9	1	76.5%	50.1%	93.2%	100.0%	95.3%	100.0%
4	64	175	239	75	31.4%	13	2	85.3%	75.3%	92.4%	96.3%	92.1%	98.6%
5	22	152	174	38	21.8%	16	1	97.4%	86.2%	99.9%	94.8%	89.6%	97.9%
6	80	35	115	29	25.2%	11	3	96.6%	82.2%	99.9%	98.8%	93.5%	100.0%
Total	373	418	791	201	25.4%	65	10	88.6%	83.3%	92.6%	96.7%	94.9%	98.0%

Note Site 6 had 3 missing BAP intrapartum culture results

Turnaround Time Analysis

The turnaround time for the 390 intrapartum subjects enrolled in the clinical trial are described below. The mean turnaround time from the time of Xpert GBS initial run-start to result-reporting was 1.84 hours (all 390 subjects). The mean turnaround time for the 360 subjects that gave valid results on first attempt was 1.76 hours. The mean turnaround time for the 30 subjects that gave valid results on second attempt was 2.74 hours. The median turnaround time for 360 specimens that gave valid results on first attempt was 1.47 hours, and 2.44 hours for the 30 subjects that gave valid results on second attempt.

Table 6. Turnaround Time from Initial Run Start to GBS Result Reporting 390 Intrapartum Subjects

	Overall	Result on First Attempt	Result on Second Attempt
Mean (hours)	1.84	1.76	2.74
Median (hours)	1.48	1.47	2.44

Analytical Performance

Analytical Sensitivity

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, VI, 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.[11] Quantitated cultures were tested in four replicates. Table 7 shows the lowest concentration of each subtype resulting in a positive result in all four replicates.

Table 7. Limit of Detection Obtained for each Serotype Tested

Serotype	CFU/Swab
ATCC12973(II)	250
Ia/c	250
Ib/c	250
II	250
III	250
IV	250
IVc	250
V	250
VI	250
VII	250
VIII	250

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 × 10^5 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.

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.

Table 8. Summary of Reproducibility Results

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

a. Expected range of Ct values; all values were within expected ranges.

Appendix

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Cepheid Headquarters Locations

Corporate Headquarters

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

European Headquarters

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

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US

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en_US/support/contact-us.

Table of Symbols

Symbol	Description
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
EC REP	Authorized Representative in the European Community
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
	Warning
Rx only	For prescription use only

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089
USA

Telephone: + 1 408 541 4191
Fax: + 1 408 541 4192
www.cepheid.com

EC REP

Cepheid Europe SAS
Vira Solelh
81470 Maurens-Scopont
France

Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

IVD

Revision History

Description of Changes: 302-9611 Rev. A

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