GeneXpert
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# Xpert® GBS

REF GXGBS-100N-10

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*In Vitro* Diagnostic Medical Device IVD CE

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# Xpert®GBS

*In Vitro* Diagnostic Medical Device

## 1 Proprietary Name

Xpert®GBS

## 2 Common or Usual Name

Xpert GBS Assay

## 3 Intended Use

The Cepheid Xpert GBS performed on the GeneXpert® Dx System is a qualitative in vitro diagnostic test designed to detect Group B Streptococcus (GBS) DNA from vaginal/rectal swab specimens, using fully automated real-time polymerase chain reaction (PCR) with fluorogenic detection of the amplified DNA. Xpert GBS Assay testing is indicated for rapid identification of antepartum and intrapartum GBS colonization.

- The use of the Xpert GBS for intrapartum screening should not preclude the use of other strategies (e.g., antepartum testing). Intrapartum Xpert GBS results are useful to identify candidates for intrapartum antibiotic prophylaxis when administration of intravenous antibiotics is not delayed pending results.
- The Xpert GBS assay does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

## 4 Summary and Explanation

GBS bacterial infection is associated with serious illness in newborns born to women who are colonized with the microorganism. 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.[1,2,3]

Currently, the standard of care for preventing neonatal GBS disease is screening pregnant women at 35–37 weeks of gestation to determine their GBS colonization status.[2] 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.[5] 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.

## 5 Principle of the Procedure

The GeneXpert Dx 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 *GeneXpert Dx System Operator Manual.*

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

After collecting and transporting a swab sample to the GeneXpert testing area, the swab is inserted into the Xpert GBS cartridge. The GeneXpert Dx System performs sample preparation by eluting the specimen material from the swab, mixing the sample reagent with the SPC (*Bacillus globigii* in the form of a bead within the cartridge) and treatment reagent, capturing cellular material on a filter, lysing the cells, and eluting the DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the integrated reaction tube for real-time PCR and detection. The results are interpolated by the GeneXpert Dx 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.

# 6 Reagents

## 6.1 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 Assay Cartridges with integrated reaction tubes** | **10** |
| • 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** | **1 per kit** |
| • Assay Definition file (ADF) | |
| • Instructions to import ADF into GX 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.

# 7 Storage and Handling

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

# 8 Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer, barcode wand reader, and 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)

# 9 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)

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## 10 Warnings and Precautions

- 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[6] and the Clinical and Laboratory Standards Institute.[7]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert GBS Assay 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 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 2-28º C

## 11 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.

## 12 Chemical Hazards[10,11]

- 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

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- **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.

# 13 Procedure

## 13.1 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. See Figure 1.
   - 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.

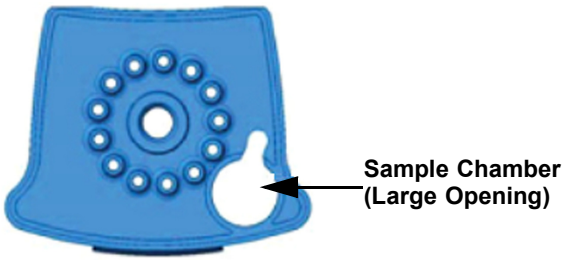


**Figure 1. Xpert GBS Cartridge (Top View)**

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### 13.2 Starting the Test

This section lists the basic steps of running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual.*

1. Turn on the GeneXpert Dx instrument and then turn on the computer, the GeneXpert software will launch automatically.
2. Log on to the GeneXpert Dx System software using your user name and password.
3. In the GeneXpert Dx System window, click **Create Test**. The Scan Cartridge Barcode dialog box appears.
4. Scan the barcode on the Xpert GBS cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
5. In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.
6. Click **Start Test**. In the dialog box that appears, type your password.
7. Open the instrument module door with the blinking green light and load the cartridge.
8. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
9. Wait until the system releases the door lock before opening the module door and removing the cartridge.

**Note** If absolutely necessary, the sample eluate can be retrieved from the cartridge sample chamber with a transfer pipette. This eluate can be used as a back-up to the paired second swab taken from the patient for susceptibility testing by the microbiology laboratory. 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.

10. Dispose of used cartridges in the appropriate specimen waste containers according to your institution's standard practices. See Section 10, Warnings and Precautions.

## 14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual.*

## 15 Quality Control

**CONTROL**

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

- **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 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 Dx 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 Dx 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).

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## 16 Interpretation of Results

The results are interpolated by the GeneXpert Dx 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. Examples of Xpert GBS Assay results are provided in Figure 2, Figure 3 and Figure 4.

**Table 1. GBS Results and Interpretation**

| Result | Interpretation |
|---|---|
| **POSITIVE**<br>See Figure 2. | GBS target nucleic acid is detected—presumptive for GBS colonization<br>• SPC—NA (not applicable)<br>• IC—NA (not applicable)<br>• Probe check—PASS<br>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**<br>See Figure 3. | GBS target nucleic acid is not detected—presumed not colonized for GBS.<br>• GBS—NEG<br>• SPC—PASS<br>• IC—PASS<br>• Probe Check—PASS |
| **INVALID**<br>See Figure 4. | 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.<br>• GBS—INVALID<br>• SPC—FAIL*<br>• IC—FAIL*<br>• Probe Check—PASS<br>* 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.<br>• GBS—NO RESULT<br>• SPC—NO RESULT<br>• IC—NO RESULT<br>• Probe Check—FAIL*<br>* 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.<br>• GBS—NO RESULT<br>• SPC—NO RESULT<br>• IC—NO RESULT<br>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.

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Figure 2 shows the GeneXpert Dx System View Results window. Click the **Errors** tab to view the error descriptions.

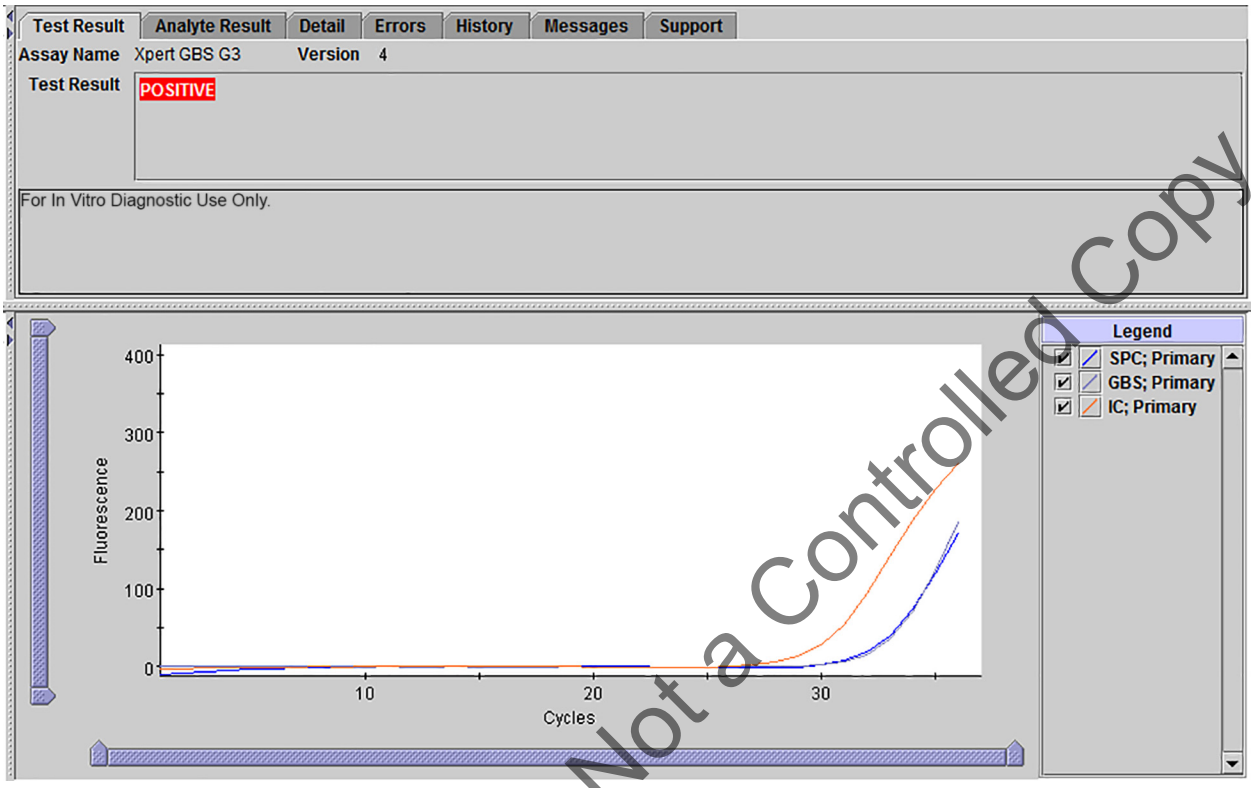


**Figure 2. GeneXpert Dx System — View Results Window Showing Positive Result**

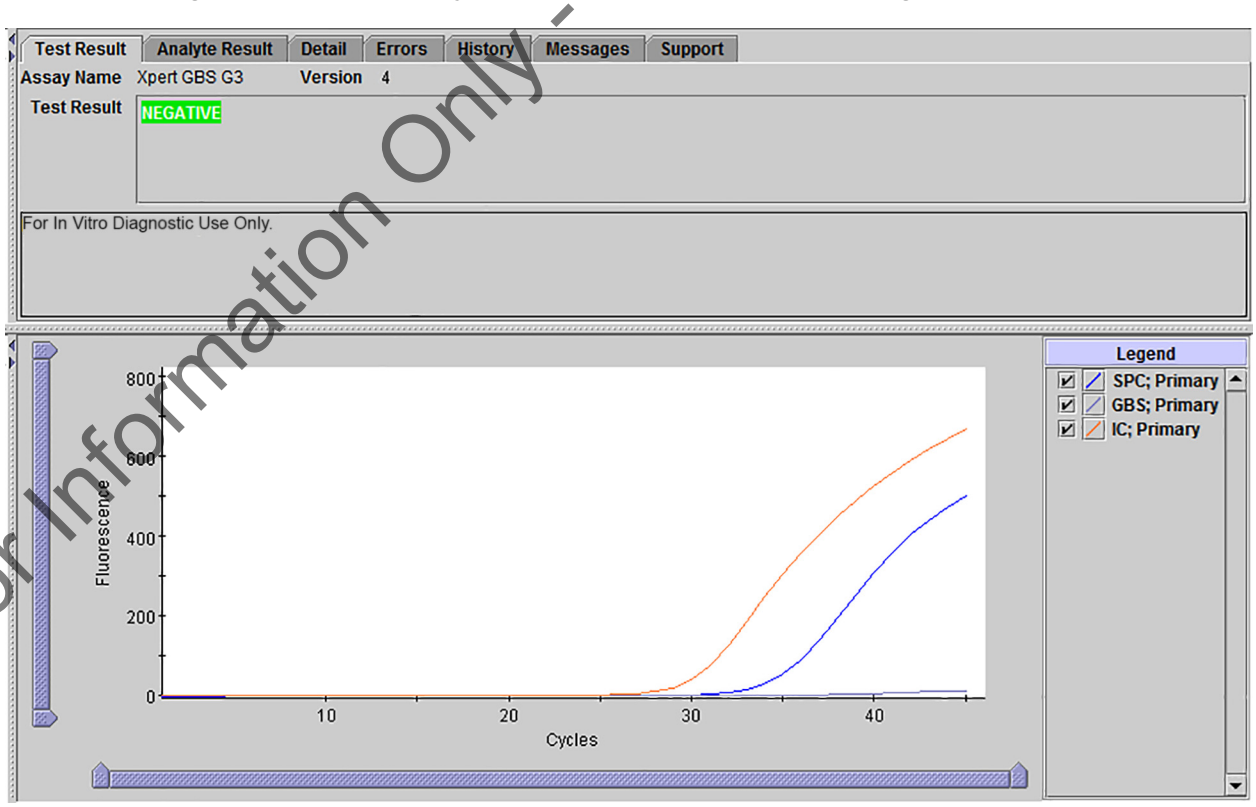


**Figure 3. GeneXpert Dx System — View Results Window Showing Negative Result**

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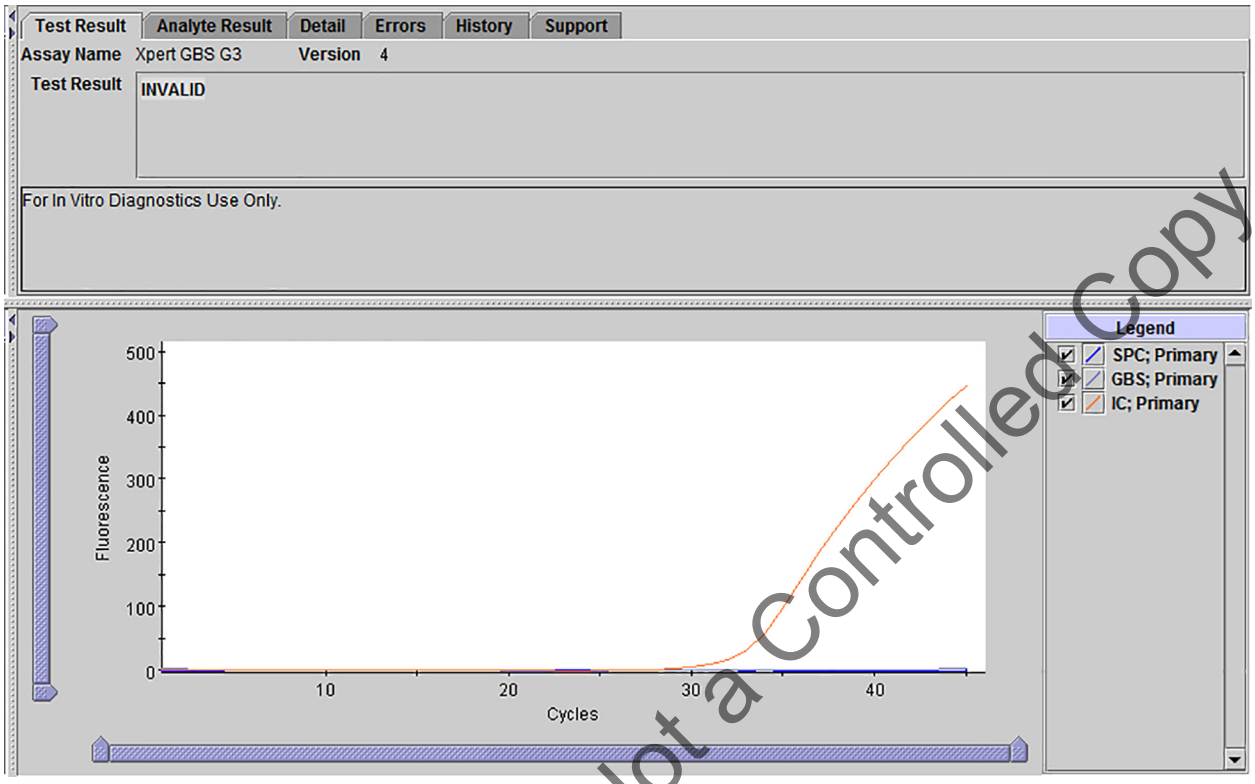


**Figure 4. GeneXpert Dx System — View Results Window Showing Invalid Result**

## 17 Reasons to Repeat the Assay

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.
- **IVALID**—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.

## 18 Limitations

- The performance of Xpert GBS Assay 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). This product can only be used with the GeneXpert Dx System. The use of specimen collection and transport system other than those listed in Section 8, Materials Required but Not Provided section is not recommended. The use of the Xpert GBS Assay 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.

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- Training for personnel using the GeneXpert Dx System is important to assure accurate and timely results.
- Rerunning the Xpert GBS Assay 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 assay 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).

## 19 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.[2,3] 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.[8] During clinical evaluations for the Xpert GBS assay, 25.4% (201 of 791) women were colonized with GBS by culture methods.

## 20 Performance Characteristics

### 20.1 Clinical Performance

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

Each institution used the culture technique recommended in the 2002 CDC guidelines[2]: 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.

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### 20.2 Overall Results

The performance characteristics of the Xpert GBS Assay 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 assay yielded 726 reportable results on the first attempt (91.8%). There were 65 non-reportable results (i.e., invalid, error, or no result); 55 of these cases resolved upon repeat testing. Overall, 201 women had cultures positive for GBS, either from the single swab used for culture or the eluted swabs from Xpert GBS and 2nd NAAT testing. The Xpert GBS assay yielded 168 positive results initially (168/201, 83.6%). After repeat testing, the positive results increased to 178/201, or 88.6%. 590 women had negative cultures and 520 were negative initially with Xpert GBS testing (88.1%), and 561 after repeat testing (95.1%).

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[1] | Culture Positive Patients[1] | Culture Negative Patients[1] | Sensitivity after Repeat Testing [95%Confidence] | Specificity after Repeat Testing [95%Confidence] | PPV [7] after Repeat Testing [95%Confidence] | NPV [8] after Repeat Testing [95%Confidence] |
|---|---|---|---|---|---|---|---|---|
| **All Patients** | Xpert GBS Pos | 197 (186) | 178 (168) | 19 (18) | 88.6% [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[2] | 10 (65) | 0 (13) | 10 (52) | | | | |
| | Total | 791[3] | 201[4] | 590 | | | | |
| **Antepartum** | Xpert GBS Pos | 92 (88) | 87 (83) | 5 (5) | 85.3% [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[2] | 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[2] | 3 (33) | 0 (7) | 3 (26) | | | | |
| | Total | 418 | 99 | 319 | | | | |
| **ROM[5]** | 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[2] | 0 (13) | 0 (2) | 0 (11) | | | | |
| | Total | 129 | 23 | 106 | | | | |
| **No ROM[6]** | Xpert GBS Pos | 78 (74) | 70 (66) | 8 (8) | 92.1% [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[2] | 3 (20) | 0 (5) | 3 (15) | | | | |
| | Total | 289 | 76 | 213 | | | | |

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

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**Table 3. Performance of Xpert GBS and 2nd NAAT Test[1] Relative to Culture**

| | Xpert GBS | | | | | | 2nd NAAT[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Category** | **Sensitivity** | **Lower CI** | **Upper CI** | **Specificity** | **Lower CI** | **Upper CI** | **Sensitivity** | **Lower CI** | **Upper CI** | **Specificity** | **Lower CI** | **Upper CI** |
| **Overall** | 88.6% (178/201) | 83.3% | 92.6% | 96.7% (561/580) | 94.9% | 98.0% | 77.9% (155/199) | 71.5% | 83.5% | 96.3% (567/589) | 94.4% | 97.6% |
| **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[2]** | 91.3% (21/23) | 72.0% | 98.9% | 94.3% (100/106) | 88.1% | 97.9% | 90.9% (20/22) | 70.8% | 98.9% | 95.2% (100/105) | 89.2% | 98.4% |
| **No ROM[3]** | 92.1% (70/76) | 83.6% | 97.0% | 96.2% (202/210) | 92.6% | 98.3% | 78.7% (59/75) | 67.7% | 87.3% | 95.8% (204/213) | 92.1% | 98.0% |

[1] The 2nd NAAT targets a sequence in the cfb gene and was previously FDA-cleared.
[2] Subset of intrapartum women who had specimens collected after membrane rupture (rupture of membrane, ROM).
[3] Subset of intrapartum women who had specimens collected before membrane rupture (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** | 165 | 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 | Prevalence | Xpert GBS with No Results | | Sensitivity | | | Specificity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Initially | Repeating | 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

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## 21 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 |

## 22 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.

## 23 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.[9] 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 |
|---|---|
| ATCC 12973 (II) | 250 |
| Ia/c | 250 |
| Ib/c | 250 |
| II | 250 |
| III | 250 |
| IV | 250 |
| IVc | 250 |
| V | 250 |
| VI | 250 |
| VII | 250 |
| VIII | 250 |

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## 24 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)[1] | 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% |

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

## 25 References


1. Schrag et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. NEJM. 2002; 247(4): 233-239.
2. Centers for Disease Control and Prevention. Prevention of Perinatal Group B Streptococcal Disease. MMWR 2002; 51 (No. RR-11): 1-26.
3. Schuchat A. Epidemiology of Group B Streptococcal Disease in the United States: Shifting Paradigms. Clin Micro Rev. 1998; 11(3): 497-513.
4. Davis et al. Multicenter Study of a Rapid Molecular-Based Assay for the Diagnosis of Group B Streptococcus Colonization in Pregnant Women. C. Infectious Disease. 2004; 30: 1129-35.
5. Puopolo et al. Early-Onset Group B Streptococcal Disease in the Era of Maternal Screening. Pediatrics. 2005; 115:1240-1246.
6. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.
7. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to latest edition).
8. Yancey MK, Schuchat A, Brown LK, Ventura VL, Markenson GR. The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. Obstet Gynecol 1996; 88: 811-15.
9. Paoletti, Lawrence C., Ph.D. Research Interests. Accessed 07/19/2006. < http://www.channing.harvard.edu/paoletti.htm>. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
10. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
11. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).


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## 26 Cepheid Headquarters Locations

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- Error messages (if any)
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## 28 Table of Symbols

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | *In vitro* diagnostic medical device |
|  | Do not reuse |
|  | Batch code |
|  | Consult instructions for use |
|  | Caution |
|  | Manufacturer |
|  | Country of manufacture |
|  | Contains sufficient for <n> tests |
|  | Control |
|  | Expiration date |
|  | CE marking – European Conformity |
|  | Temperature limitation |
|  | Biological risks |
|  | Warning |
|  | Authorized Representative in the European Community |
|  | Authorized Representative in Switzerland |
|  | Importer |

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