Policy Statement	Laboratory personnel are responsible for insuring the specimen submitted for testing is acceptable and adhering to the procedure for performing the test.
Purpose	This procedure provides technical instruction for the performance of the Xpert™ GBS Assay.
Scope	This procedure applies to testing personnel authorized to perform PCR testing. This group includes, but is not limited to Medical Technologists as well as leads and supervisory personnel.
Responsibility	All the above personnel are responsible for following the Xpert™ GBS Assay procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
Related Documents	CORE 6610 J Xpert™ GBS Assay Quick Reference CORE 6600 R GeneXpert Quality Control and Maintenance

Specimen Collection

In order to obtain an adequate specimen, the procedure for specimen collection must be followed closely.

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. Ship the container to the laboratory according to the hospital standard operating procedures.

Specimen Handling and Retention

Handling

Specimens should be kept between 2 °C and 25 °C during transport. Protect against freezing or exposure to excessive heat.

Specimens can be stored up to 6 days at 2-8 °C before testing. Specimens can be kept at room temperature (15-25 °C) for up to 24 hours before testing.

Retention

Once a sample has arrived in the laboratory the following requirements must be met to ensure specimen integrity.

- All samples should be received in the laboratory module of Meditech.
- All samples should be retained in the appropriate rack in the refrigerator until tested to reduce chance of specimen loss.

- Samples should not be opened until testing is performed to eliminate chance for contamination or alteration.
- A pending log should be checked to verify that all samples are accounted for in the laboratory.

Reagents and Supplies

Reagents

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 (freeze-dried) 1 per cartridge

- Polymerase/inhibitor complex
- BSA (bovine serum albumin)

Bead 2 (freeze-dried) 1 per cartridge

- Primers
- Probes
- dNTPs
- Internal control DNA (IC)
- BSA (bovine serum albumin)

Bead 3 (freeze-dried) 1 per cartridge

- Sample Processing Control (SPC) (~2000 B. globigii spores) preparation control spores

Reagent 1 Elution buffer 10 x 3.0 mL
Reagent 2 Treatment Reagent 10 x 3.0 mL

External Controls

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)

Reagent Guidelines

-  Store the Xpert™ GBS Assay cartridges and reagents at 2–28 ºC.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge and reagents within 2 hours after opening the package.
- Do not use any reagents that have become cloudy or discolored.

Supplies

- GeneXpert Dx System with software
- Collection Swabs (Liquid Stuarts; white cap single swab or red cap dual swab) Swabs with gel are not acceptable.
- Disposable gloves, powderless
- Gauze
- Sterile pipette
- Sterile tweezers

Quality Control

Each test includes the following internal controls and a probe check.

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—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 and Lot Verifications —External controls are used in accordance with local, state, federal accrediting organizations, as applicable. See *CORE 6600 GeneXpert Quality Control and Maintenance* for details.

Procedure

Preparing the Cartridge

Important: Start the test within 15 minutes of adding the reagents to the cartridge.

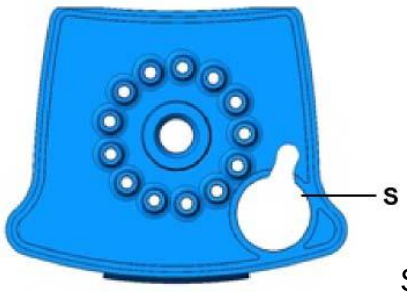
Note: If multiple swabs are received, only one swab is required. Do not add 2 swabs to any one cartridge

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

1. Remove the cartridge and reagents from the package.
2. Label the cartridge with the sample ID.
3. Open the cartridge lid.
4. Remove the marked swabs from the container. Gently rub the two swabs together using a twirling motion so that equal amount of sample is on each swab.
5. Insert one of the swabs into the Xpert GBS cartridge chamber S.
    - Do not insert both swabs into the cartridge.
    - Return the second swab into the collection/transport tube. 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.

***Note** – Keep cartridge in an upright position. Allowing the cartridge to turn on the side will cause contamination and directly affect the sample results.

Figure 1 - Xpert GBS Assay cartridge (top view)



S =Sample

CHANGE GLOVES BEFORE CONTINUING TO NEXT STEP!

Starting the Test

1. Turn on the computer, and then turn on the GeneXpert Dx instrument.
2. On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
3. In the GeneXpert Dx System window, click **Create Test**. The Patient ID barcode dialog box appears.
4. Enter the Patient's Last name in the **Patient ID Barcode** field. The Scan Cartridge Barcode dialog box appears.
5. 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.
6. In the **Sample ID** box, type the sample ID (*e.g.* SM0000). 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.
7. In the **Notes** box, scan or type your personal identification barcode. This will identify you as the person that performed the testing.
8. Click **Start Test**.
9. Open the instrument module door with the blinking green light and load the cartridge.

10. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
11. Wait until the system releases the door lock before opening the module door and removing the cartridge.

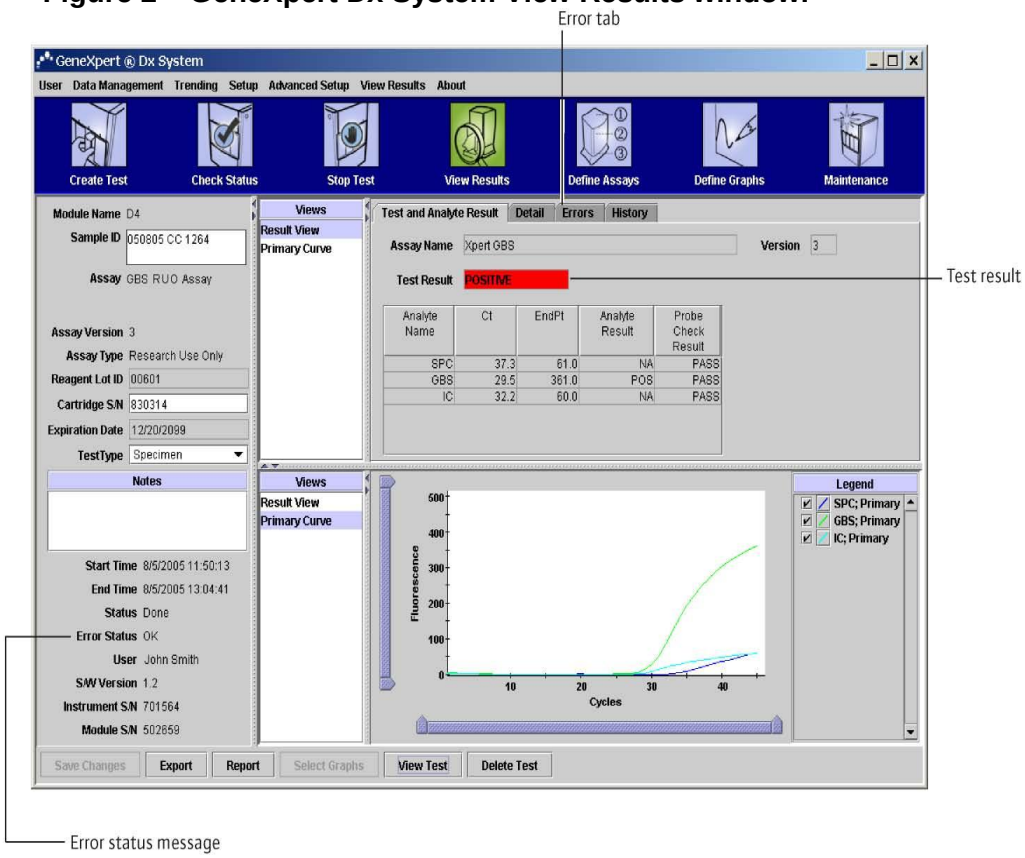
Reporting

Assay 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:

Possible Results	Description	
	Component	**Interpretation**
POSITIVE	GBS target nucleic acid is detected—presumptive for GBS colonization	
	SPC	NA (not applicable)
	IC	NA (not applicable)
	Probe Check	PASS
NEGATIVE	GBS target nucleic acid is not detected—presumed not colonized for GBS.	
	SPC	PASS
	IC	PASS
	Probe Check	PASS; all probe check results pass.
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.	
	SPC	FAIL
	IC	FAIL
	Probe Check	PASS
ERROR	Presence or absence of GBS cannot be determined. A system component failed, the maximum pressure was reached, or the probe check failed.	
	SPC	NO RESULT
	IC	NO RESULT
	Probe Check	FAIL
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.	
	SPC	NO RESULT
	IC	NO RESULT
	Probe Check	NA (not applicable)

Figure 2 – GeneXpert Dx System View Results window.



Before reporting GBS results, always verify that the assay is valid.

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.
- **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 two swabs were received, prepare a new cartridge using the second swab, and then

rerun the test. If only one swab was received and there is fluid in the cartridge S chamber, use a sterile transfer pipette to transfer all the fluid to the S chamber of a new cartridge, and then rerun the test from the Add Reagent 1 into cartridge chamber 1 step. If there is no fluid, use sterile tweezers to transfer the swab to a new cartridge, and then rerun the test from Add Reagent 1 into cartridge chamber 1.

Entering the result in Meditech

1. Review your report to ensure that all checks passed.
2. Log into Meditech and go into Enter Results on the Specimen Desktop.
3. Enter SM#, type E to go to page 1, enter organism code as **NEGGBS** for negative samples and **POSGBS** for positive samples.
4. Click on page 2 for all samples. A positive result is an alert value. If result is positive enter canned comment **SMART** and patient location with the interpretation.
5. File Result.

Refer to the SmartCycler Dx Software Operator Manual for printing of results.

Specimen Retention after Assay

If multiple swabs were received, upon completion of assay, all specimens will be discarded.

Principle

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*.

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 and two reagents are added to appropriate chambers of 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.

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.

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 the 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, not following the recommended sample-collection procedure, handling or storage, 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 Dx System is important to assure accurate and timely results.

- Culture isolates are needed for performing susceptibility testing in penicillin-allergic women. A second sample must be submitted to microbiology for testing if this service is required. Culturing from Xpert GBS Reagent 1 has not been validated.
- 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 required to avoid contamination of specimens or reagents.

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 inPregnant 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 McKinneyRW (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 predictinggenital group B streptococcal colonization at delivery. Obstet Gynecol 1996; 88: 811-15.

[9] Paoletti,LawrenceC.,Ph.D.ResearchInterests.Accessed07/19/2006.< http://www.channing.harvard.edu/paoletti.htm>.