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® C *difficile* 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® C *difficile* Assay procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
Related Documents	CORE 6605 J Xpert® C *difficile* Assay Quick Reference CORE 6600 GeneXpert Quality Control and Maintenance MICR 6011 J Critical Care Indicators

Specimen Collection

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

Liquid stool or soft stool specimen only

Using a dry sterile container or Cary Blair media container, liquid or soft stool specimens are collected according to the following procedure.

1. Transfer liquid or soft stool (not urine) into the container. Avoid mixing toilet paper, water, or soap with the sample.
2. Label the container.
3. 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 5 days at 2-8 °C before testing. Specimens can be kept at room temperature (15-25 °C) for up to 24 hours before testing. Specimens can be tested after one (1) freeze and thaw cycle.

Due to the nature and sensitivity of testing, only one patient sample will be tested per shift. All duplicates should be canceled and the patient care area notified.

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 bin 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 *C. difficile* Assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert *C. difficile* Assay Cartridges with integrated reaction tubes 10 (120)

Bead 1 (freeze-dried) 1 per cartridge
- Polymerase
- dNTPs
- BSA (bovine serum albumin)
- Probe

Bead 2 (freeze-dried) 1 per cartridge
- Primers
- Probes
- BSA

Bead 3 (freeze-dried) 1 per cartridge
- Sample Processing Control (SPC) non-infectious sample preparation control spores

Reagent 1 (Sodium Hydroxide) 3.0 ml per bottle per cartridge
Reagent 2 (Tris Buffer, EDTA and surfactants) 3.0 ml per bottle per cartridge

Xpert *C. difficile* Assay Reagent pouch 1
- Sample Reagent (Guanidinium thiocyanate and surfactants) 10 (125)

2.0 ml per bottle

****Do not mix the klt contents of different lot numbers.****

External Controls

KWIK-STIKs™ from MicroBioLogics catalog #0329 (toxigenic *C. difficile*) as positive control, and catalog #0527 (non-toxigenic *C. difficile*) as negative control.

Reagent Guidelines

-  Store the Xpert *C. difficile* 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 7 days of opening the package.
- Do not use any reagents that have become cloudy or discolored.

Supplies

- Dry sterile swab
- Dry sterile container or Cary Blair Media container for collection of stool sample
- Vortex
- Vortex adapter piece with multiple holding sites and retainer
- Gauze
- Disposable sterile pipettes
- Disposable gloves, powderless
- GeneXpert Dx System with software

Quality Control

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

Sample Processing Control (SPC) — Ensures the sample was correctly processed.

The SPC contains spores of *Bacillus globigii* in the form of a dry spore cake that is included in each cartridge to verify adequate processing of the sample bacteria. The SPC verifies that lysis of *C. difficile* bacteria and spores has occurred; if the organisms are present, and verifies that specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the real-time PCR assay. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated 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 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 30 minutes of adding the reagents and sample to the cartridge.
To add the sample and reagents to the cartridge:

1. Remove the cartridge and reagents from the package.

2. Label the cartridge with the sample ID.

3. Briefly place a swab in the liquid or soft stool sample. The swab does not need to be completely saturated. Swirl off excess stool.

4. Insert the swab into the vial containing the Sample Reagent.
Note: Use sterile gauze to minimize risks of contamination.

5. Hold the swab by the stem near the rim of the vial, lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the vial to break it. Make sure the swab is short enough to allow the cap to close tightly.

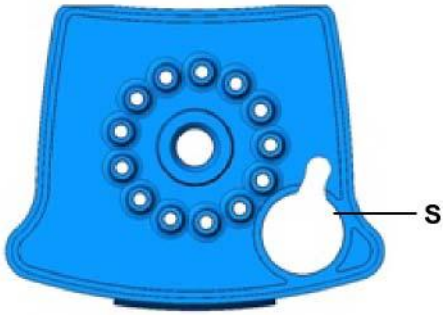
6. Close the lid and vortex at high speed for 10 seconds.

7. Open the cartridge lid. Using a sterile transfer pipette, transfer the entire contents of the Sample Reagent to the “S” chamber of the Xpert *C. difficile* Assay cartridge.

8. 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 *C. difficile* 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. Barcode dialog box appears.
5. Scan the barcode on the Xpert *C. difficile* Assay 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. In the dialog box that appears.

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. Forcing the door to open before the system releases the lock could result in a system jam.
12. Dispose of the used cartridges in an appropriate specimen waste container according to your institution's standard practices.

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**
Toxigenic C *difficile* POSITIVE	Toxin producing C. *difficile* target DNA sequences are detected.	
	SPC	NA; SPC is ignored since C *difficile* target amplification may compete with this control
	Probe Check	PASS; all probe check results pass.
Toxigenic C *difficile* NEGATIVE	C. *difficile* target DNA sequences are not detected.	
	SPC	PASS; SPC has a Ct within in the valid range and endpoint above the endpoint minimum setting.
	Probe Check	PASS; all probe check results pass.
INVALID	Presence or absence of C. *difficile* cannot be determined.	
	SPC	FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint minimum setting.
	Probe Check	PASS; all probe check results pass.
ERROR	Presence or absence of C. *difficile* cannot be determined.	
	Toxin producing C. *difficile* targets – NO RESULT	
	Probe Check	FAIL*; one or more of the probe check results fail.
NO RESULT	Presence or absence of C. *difficile* cannot be determined.	
	Toxin producing C. *difficile* targets – NO RESULT	
	Probe Check	NA (not applicable)

* If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range.

Figure 2 - An example of a toxigenic *C. difficile* POSITIVE result.

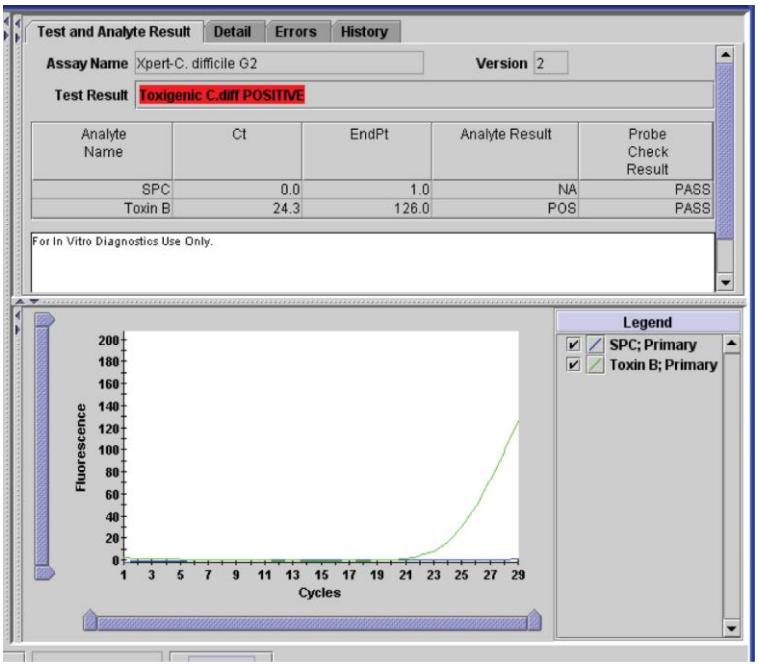


Figure 3. An example of a toxigenic *C. difficile* NEGATIVE result.

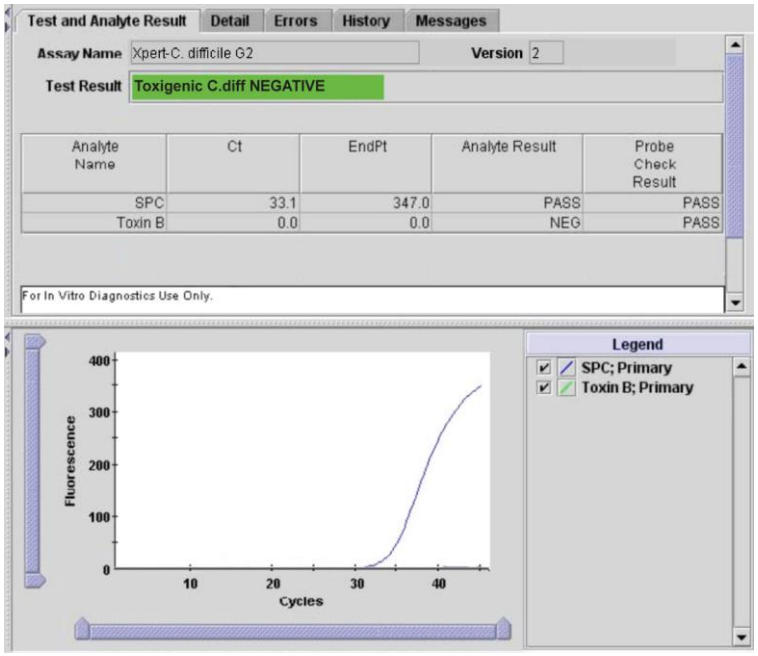
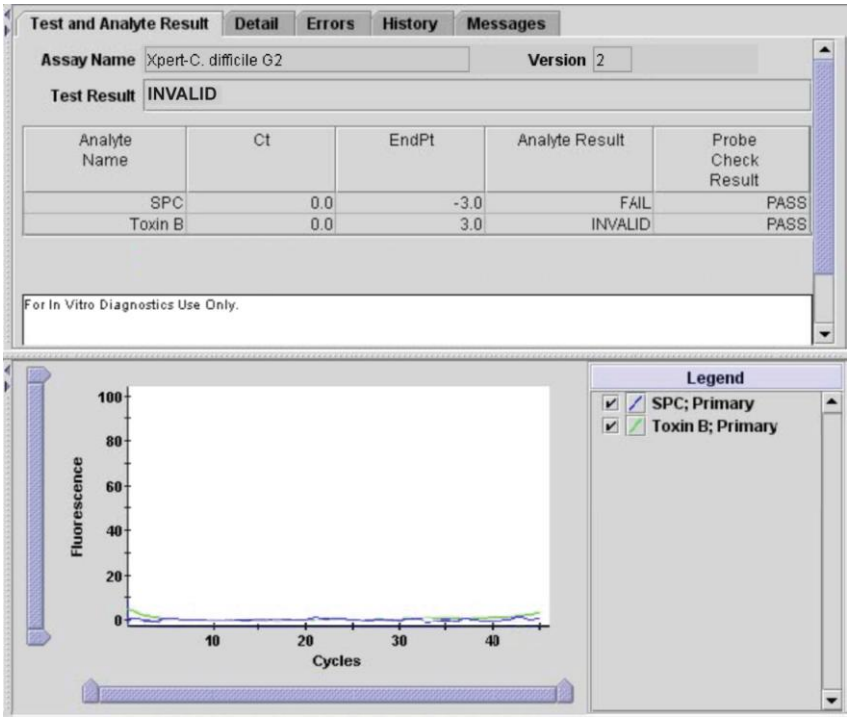


Figure 4. An example of an INVALID result.



Invalid Controls

An invalid SPC or Probe Check the sample results. In such cases, assay results obtained for that run are invalid and must not be reported. Invalid assay run or instrument error codes or warnings are flagged on-screen and on reports. Before reporting *C. difficile* results, always verify that the assay is valid.

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

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 2, enter organism code as **NEGCDT** for negative samples and **POSCDT** for positive samples.
4. Click on page 3 for all samples. If result is positive enter canned comment **SMART** and the patient location with the interpretation.

5. File Result.

Reporting Infection Control Value

Positive results are considered Significant Infection Control Values. All positive results must be added to the Meditech Critical Care Indicator Application. See *MICR 6011 J Critical Care Indicators* for details.

Specimen Retention after Assay

Upon completion of assay, all specimens will be retained. Specimens will be retained in Microbiology for one week.

Principle

The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. For a full description of the system, see the GeneXpert Dx System Operator Manual.

The Xpert *C. difficile* Assay includes reagents for the detection of toxigenic *C. difficile*, as well as a Sample Processing Control (SPC). The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The Cepheid Xpert *C. difficile* assay is a rapid, automated *in vitro* diagnostic test for

qualitative detection of toxin producing *Clostridium difficile* directly from unformed (liquid or soft) stool specimens of patients suspected of having *Clostridium difficile* infection (CDI). The assay detects the toxin B gene (*tcdB*). The assay is performed on the Cepheid GeneXpert® Dx System.

Intended Use

The Cepheid Xpert® *C. difficile* Assay, performed on the Cepheid GeneXpert® Dx System, is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The test utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile* Assay is intended as an aid in the diagnosis of CDI. Concomitant culture is necessary only if further typing or organism recovery is required.

Limitations

- This test detects but does not differentiate the NAP1 (Ribotype 027) strain from other toxigenic strains of *C. difficile*.
- This test targets the *tcdB* gene for Toxin B production. This test will not detect strains of *C. difficile* that do not contain the *tcdB* gene.
- The performance of the Xpert *C. difficile* Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Results from the Xpert *C. difficile* Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because of the dilution factor associated with the retest procedure, it is possible that *C. difficile* positive specimens, very near or at the limit of detection (LoD) of the Xpert

C. difficile Assay, may result in a false negative result upon retest.

- Inhibition of the Xpert *C. difficile* Assay has been observed in the presence of the following substances: Zinc oxide paste and Vagisil® cream.

Interfering Substances

Twenty-one (21) biological and chemical substances occasionally used or found in stool specimens were tested for interference with the Xpert *C. difficile* Assay. Potentially interfering substances include, but are not limited to, Vagisil cream and zinc oxide paste. The 19 substances listed in Table 5 of the package insert showed no detectable interference with the Xpert *C. difficile* Assay.

References

1. Cepheid GeneXpert Dx System Operator Manual
2. Clinical and Laboratory Standards Institute. Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline, document MM3-A2 (Refer to the latest edition).
3. Package Insert - Xpert® C *difficile* Assay (300-7871 Rev.A)

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.

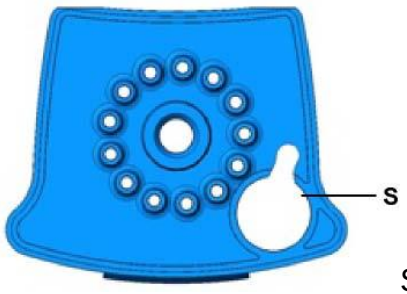
Note: If multiple swabs are received, only one swab is required. Do not add 2 swabs to any one cartridge

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.

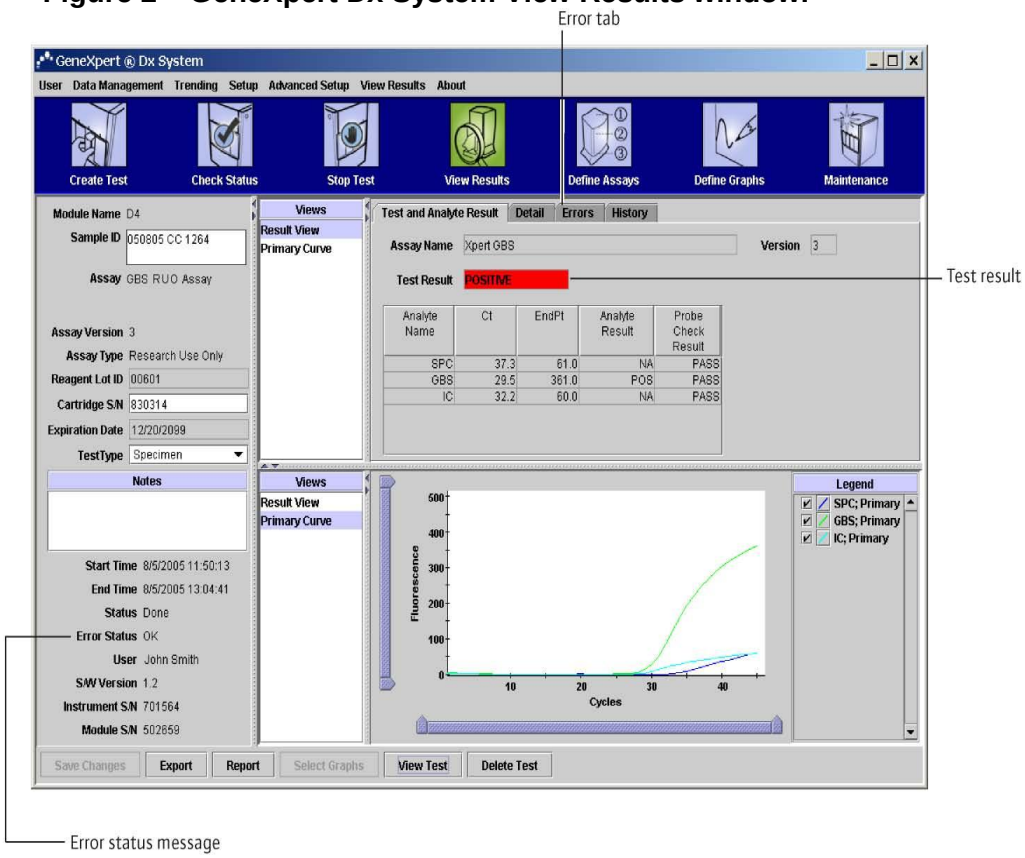
Reporting

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>.

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™ EV Assay.
Scope	This procedure applies to technical 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™ EV Assay procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.

Specimen Handling and Retention

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

Cerebrospinal Fluid (CSF) only

Using a dry sterile container, collect CSF according to the standard procedures.

1. Transfer liquid to container.
2. Label the container
3. Transport specimen to the laboratory.

Handling

Specimens must be kept at 2-8 ˚C until testing. Specimen should be protected from excessive heat.

Specimens can kept at 2-8 ˚C for up to 72 hours.

Centrifugation of the specimen is not recommended. Due to the nature of the specimen, a test will not be cancelled if the only sample available has been centrifuged.

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 EV assay kit (GXEV-100N-10) contains sufficient reagents to process 10 samples. The kit contains the following:

Xpert EV Cartridges	10 cartridges/kit
Bead 1 (freeze-dried)	1 per cartridge
•Reverse transcriptase	
•RNase Inhibitor	
•dNTPs	
•BSA (bovine serum albumin)	
Bead 2 (freeze-dried)	1 per cartridge
•Reverse Oligonucleotide Primers	
•BSA (bovine serum albumin)	
Bead 3 (freeze-dried)	1 per cartridge
•DNA Polymerase	
•dNTPs	
•BSA (bovine serum albumin)	
Bead 4 (freeze-dried)	1 per cartridge
•Forward Oligonucleotide Primers	
•Fluorescently labeled Oligonucleotide Probes	
•BSA (bovine serum albumin)	
Bead 5 (freeze-dried)	1 per cartridge
•Sample processing control (encapsidated CIC RNA pseudovirus)	
•BSA (bovine serum albumin)	
Binding Reagent (1)	10 × 1 mL
•Ethanol	
•Water	
Wash Reagent (2)	10 × 3.2 mL

- Cysteamine HCl
- EDTA (ethylenediaminetetraacetic acid)
- KCl
- MTG (monothioglycerol)
- PEG (polyethyleneglycol)
- Sodium azide 0.05% w/v
- Tris, pH 7.0
- Tween-20
- Water

Elution Reagent (3) — 10 × 2.0 mL

- Ammonium sulfate
- EDTA (ethylenediaminetetraacetic acid)
- Sodium azide 0.05% w/v
- Tris, pH 7.0
- Water

Lysis Reagent (4) — 10 × 300 µL

- N-acetyl-L-cysteine
- Guanidine thiocyanate
- N-lauroylsarcosine
- Sodium citrate, pH 6.8
- Water

****Do not mix the klt contents of different lot numbers.****

External Controls

Acrometrix® OptiQual Controls - Cat. no. 950500 and 950499: EV Low Control contains inactivated human coxsackie virus particles from strain CVA9, EV Negative Control contains only OptiMatrix Clear (a synthetic matrix designed to mimic naturally occurring human cerebrospinal fluid specimens)

Reagent Guidelines

- Store the Xpert EV cartridges and reagents at 2-28 ºC.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge and reagents within 30 minutes after opening the package.
- Do not use cartridges or reagents that have passed the expiration date.
- Do not use any reagents that have become cloudy or discolored.
- Material Safety Data Sheets (MSDS) for all reagents provided in the testing area.
- The bovine serum albumin (BSA) in this product was produced exclusively from bovine plasma sourced in the United States. The manufacturing of the BSA is also performed 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 commingling of the material with other animal materials.

Supplies

- GeneXpert® Dx System
- Xpert EV Assay CD
- Printer
- 200-µL pipette
- Disposable sterile 200-µL pipette tips

Warnings and Precautions

- For *In Vitro* Diagnostic Use Only.
- Over a period of time, sodium azide might react with copper, lead, brass, or solder in plumbing systems to form an accumulation of the highly explosive compounds of lead azide and copper azide.[5]
- Lysis Reagent contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If liquid containing this reagent is spilled, clean the area with laboratory detergent and water.
- Treat all biological specimens, including cartridges, as if they are capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all human specimens should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[6] and the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards).[7]

- Dispose of all hazardous or biologically contaminated materials according to standard practices. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Quality Control

Each test includes two internal controls to validate the assay: Sample-processing control/internal control and probe check. Test samples are controlled according to the following procedures:

Sample-processing control/internal control (SPC/IC)—The SPC/IC is an encapsidated RNA pseudovirus in the form of a dry bead and is included in each cartridge. The SPC/IC verifies adequate lysis of target EV and sample processing, and detects assay interference. It is mixed with the sample to control for adequate sample processing and to monitor the integrity of the RT-PCR assay. The SPC/IC is considered to pass if it meets the validated acceptance criteria. Note that in the GeneXpert® Dx System software, CIC is the name for the SPC/IC.

Probe check—Before the start of the PCR reaction, the system performs a probe check on both the EV target and the SPC/IC to verify reagent bead rehydration and reaction-tube filling. Each probe check is considered to pass if it meets the validated 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

To add the sample and reagents into the cartridge (See Figure 1):

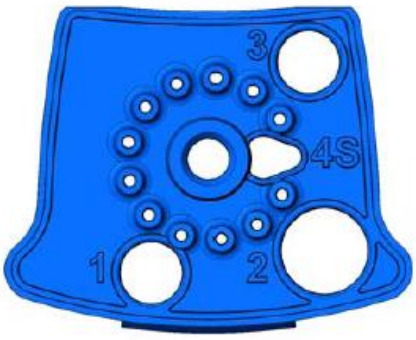
1. Remove a cartridge and the reagents from the package.

2. Label the cartridge with the sample ID.

3. Open the Binding Reagent (1) ampule by twisting and breaking off the cap.

4. Insert the tip of the Binding Reagent (1) ampule into cartridge chamber 1 and squeeze the ampule until the entire content is emptied.
5. Open the Wash Reagent (2) ampule by twisting and breaking off the cap.
6. Insert the tip of the Wash Reagent (2) ampule into cartridge chamber 2 and squeeze the ampule until the entire content is emptied.
7. Open the Elution Reagent (3) ampule by twisting and breaking off the cap.
8. Insert the tip of the Elution Reagent (3) ampule into cartridge chamber 3 and squeeze the ampule until the entire content is emptied.
9. Using the 200-µL pipette, add 140 µL of the Lysis Reagent (4) to cartridge chamber 4S. Discard the Lysis Reagent (4) vial.
10. Using the 200-µL pipette, add 140 µL of the sample to cartridge chamber 4S. To prevent large air bubbles from forming, be sure to hold the pipette tip at the top of the chamber and dispense the sample slowly.
11. Close the cartridge lid.

Be sure to load the cartridge into the GeneXpert® Dx instrument and start the test within 30 minutes of adding the reagents.



1 = Binding Reagent
2 = W ash Reagent
3 = Elution Reagent
4S = Lysis Reagent and Sample

Figure 1. Xpert EV cartridge (top view).

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 bar code on the Xpert EV cartridge. The **Create Test** window appears. Using the bar code information, the software automatically fills the following boxes: Select Assay, Reagent Lot ID, Cartridge S/N, and Expiration Date.
6. In the **Sample ID** box, type the sample ID (*e.g.* SM1234). 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 module door. Be sure the green light is solid green.
11. When the test is finished, the instrument module light turns off.
12. Wait until the system releases the door lock before opening the module door and removing the cartridge.
13. Dispose of the used cartridge in the appropriate specimen waste container.

Cautions

- Do not substitute Xpert EV reagents with other reagents.
- Do not open the Xpert EV cartridge lid except when adding sample and reagents.
- Do not load a Xpert EV cartridge that has been dropped or shaken after you have inserted the sample and reagents.
- Do not load a cartridge that has a damaged reaction tube.
- Do not open used Xpert EV cartridges.
- ⊘ Do not reuse spent Xpert EV cartridges.
- Do not freeze and thaw the specimens more than two times.
- Do not use specimens that have been centrifuged.

Reporting

Assay Results

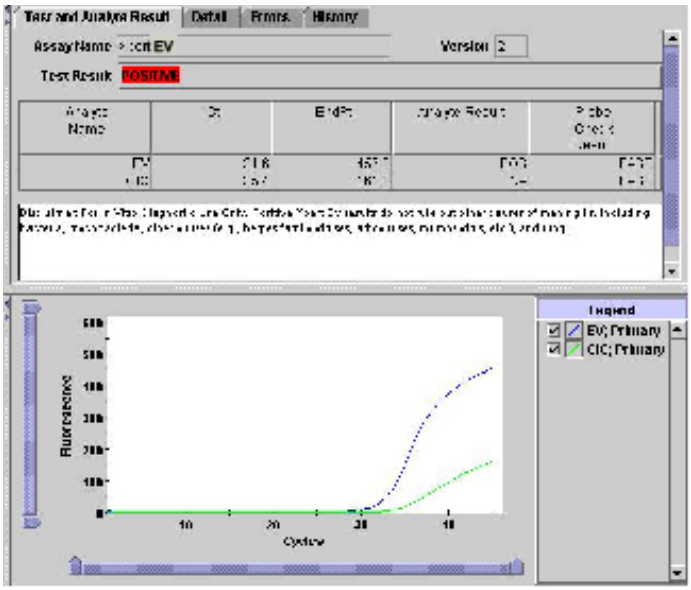
The results are interpolated by the GeneXpert Dx System from measured fluorescent signals and embedded calculation algorithms and will be shown in the **View results** window. Possible results are:

Possible Results	Description	
	Component	**Interpretation**
EV POSITIVE	EV target nucleic acid is detected.	
	SPC	NA (When EV titer is high, the RT-PCR for the SPC might be suppressed)
	Probe Check	PASS; all probe check results pass.
EV NEGATIVE	EV target nucleic acid is not detected.	
	SPC	PASS; SPC has a Ct within in the valid range and endpoint above the endpoint minimum setting.
	Probe Check	PASS; all probe check results pass.
INVALID	Presence or absence of EV target nucleic acid cannot be determined.	
	SPC	FAIL; SPC target result is negative and the SPC Ct is not within valid range and endpoint minimum setting.
	Probe Check	PASS; all probe check results pass.
ERROR	Presence or absence of EV target nucleic acid cannot be determined.	
	EV – NO RESULT, SPC/IC (CIC) – NO RESULT	
	Probe Check	FAIL*; one or more of the probe check results fail.
NO RESULT	Presence or absence of EV target nucleic acid cannot be determined.	
	EV – NO RESULT, SPC/IC (CIC) – NO RESULT	
	Probe Check	NA (not applicable)

* If the probe check passed, the error is caused by the maximum pressure limit exceeding the acceptable range.

CAUTION: ⚠ **Positive Xpert EV results do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses (e.g. herpes family viruses, arboviruses, mumps virus, etc) and fungi.**

Figure 2. Positive Result



- **Negative Xpert EV results do not rule out enterovirus as causes of meningitis but that enterovirus was not detected.**

Figure 3. Negative Result

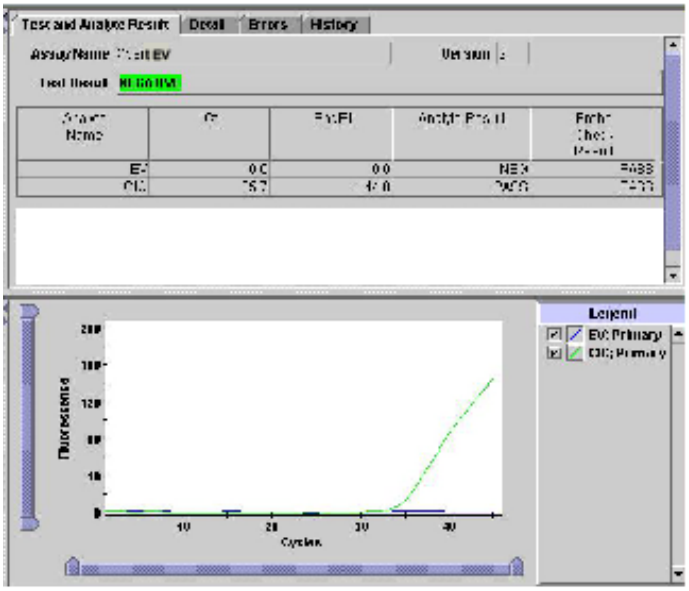
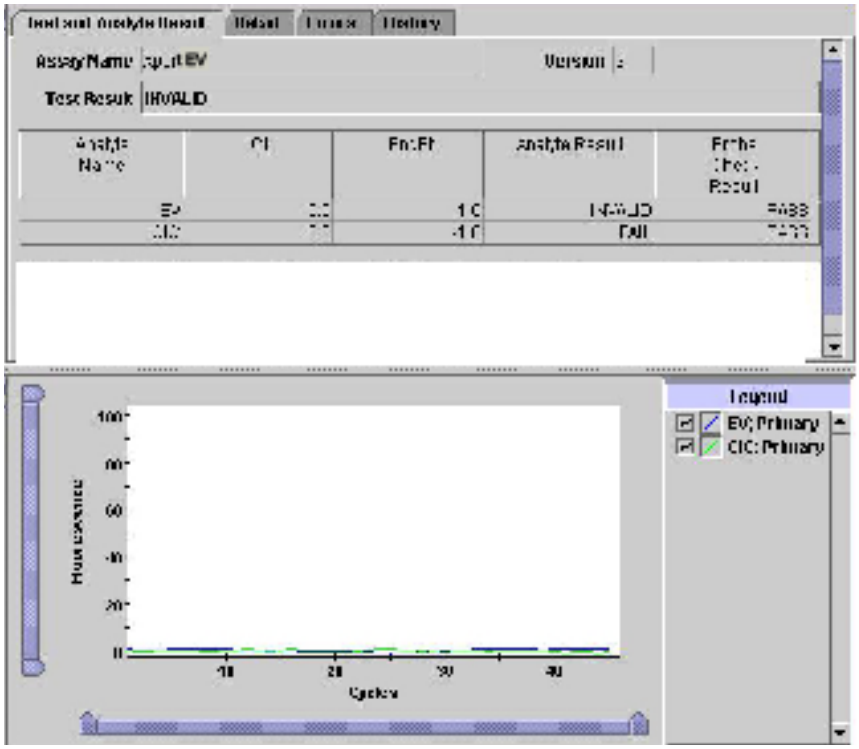


Figure 4. Invalid Result



Reasons to Repeat the Assay

Repeat the assay with fresh sample if the following results are generated:

- An INVALID result indicates that the controls SPC/IC failed. The sample was not properly processed or PCR is inhibited.
- An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.
- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Contact the patient care area prior to repeating the test to inform them of the delay in testing.

In the event that a sample result is INVALID twice, the sample should be frozen for a few hours. The sample should then be thawed and testing repeated.

Invalid Controls

An invalid SPC or Probe Check the sample results. In such cases, assay results obtained for that run are invalid and must not be reported. Invalid assay run or instrument error codes or warnings are flagged on-screen and on reports.

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

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

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 **NEGEV** for negative samples and **POSEV** for positive samples.

4. Click on page 2 for all samples. If result is positive enter canned comment **CALD** with the interpretation. Call the patient care area with the result and document the name of the person taking the result.
5. File Result.

Specimen Retention after assay

Upon completion of the assay, all specimens will be archived in the Core Laboratory and retained for seven days.

Principle of the Procedure

The GeneXpert® Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR assays. 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 Xpert single-use disposable GeneXpert® cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. For a full description of the system, see the *GeneXpert® Dx System Operator Manual*.

The Xpert EV assay is designed to detect enterovirus (EV) RNA (enterovirus genome 5' untranslated region [UTR] between nucleotide 452 and 596) in CSF samples. The assay includes reagents, primers, and probes for the simultaneous detection of nucleic acid from the target EV and the sample-processing control/internal control (SPC/IC). The assay includes the SPC/IC to verify adequate processing of the target virus and monitors the presence of inhibitors in the RT-PCR assay to avoid a false negative result. (Note that in the GeneXpert® Dx System software, CIC is the name for the SPC/IC.) The assay also includes a probe check control to verify reagent rehydration, probe integrity, and reaction-tube filling in the cartridge. To run a test, the CSF sample and four reagents are transferred into designated chambers of the Xpert EV cartridge. The GeneXpert® Dx System performs sample preparation by lysing the virus and SPC

(encapsidated RNA pseudovirus), binding the RNA to the capture matrix, and eluting the RNA. The RNA is mixed with dry RT reagents and transferred into the reaction tube for preparation of cDNA. The cDNA is then mixed with dry PCR reagents and transferred into the reaction tube for real-time PCR and detection. The EV primers and probe amplify and detect a consensus region of the enterovirus 5' untranslated region (UTR). The test takes approximately 2.5 hours.

Intended Use

The Cepheid® Xpert EV assay is a reverse transcription polymerase chain reaction (RT-PCR) using the GeneXpert® Dx System for the presumptive qualitative detection of enterovirus (EV) RNA in cerebrospinal fluid (CSF) specimens from individuals with signs and symptoms of meningitis. This test, in conjunction with other laboratory results and clinical information, may be used as an aid in the laboratory diagnosis of enterovirus infection in patients with a clinical suspicion of meningitis or meningoencephalitis. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients.

CAUTION: ⚠ The results obtained with the Xpert EV assay should be used only as an adjunct to clinical observations and other information available to the physician. Positive Xpert EV results do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses (e.g., herpes family viruses, arboviruses, mumps virus, etc.), and fungi.

Limitations

- Results from the Xpert EV assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician. An Xpert EV assay positive result does not rule out the presence of another pathogen like bacteria in CSF. As with any molecular assay, false positive results are always a possibility. Rare

occurrences of simultaneous mixed bacterial-viral meningitis have been reported in the literature.[8, 9, 10]

- The performance of the Xpert EV assay was validated using the package insert and with the Cepheid GeneXpert® Dx System only. Modifications should not be made to these procedures as they might alter the performance of the test.
- The Xpert EV assay is for the detection of enterovirus only. Negative test results do not rule out the presence of enterovirus. This test does not rule out the possibility of Herpes-induced meningitis or fungal meningitis; additional testing is required to rule out these infections.

CAUTION: ⚠ As with other diagnostic procedures, the results obtained with the Xpert EV Assay should be used only as an adjunct to clinical observation and other information available to the physician. Positive Xpert EV results do not rule out other causes of meningitis, including bacteria, mycobacteria, other viruses (e.g. herpes family viruses, arboviruses, mumps virus, etc) and fungi.

Interfering Substances

Studies were conducted with potential interfering substances encountered in CSF. Substances tested were white blood cells, protein, whole blood and hemoglobin. WBC content was tested using leukocytes (K562 human leukemia cells) spiked into CSF.

To address potential interference from bloody taps, human CSF specimens contaminated with various levels (up to 125,000 RBC/mm^3) of blood were tested.

The concentration ranges and the interfering substances found in normal CSF are indicated in Samples of potentially interfering endogenous substances tested in Xpert EV. Also indicated are the potential ranges found in CSF during meningitis. Each substance was spiked at levels that could be encountered with normal or meningitis patients.

All tests were performed with CSF spiked with enterovirus serotype CVA9 at 80 $TCID_{50}$/mL (~3x LOD).

Table 1a. Samples of potentially interfering endogenous substances tested in Xpert EV.

Substance	Concentration range found in normal CSF	Potential CSF concentration range (during meningitis)	Sample tested with Xpert EV	Concentrations Tested
WBC	0-5 cells/mm^3	5-5000 cells/mm^3	K562 cells	Cells/mm^3: 0, 3.57, 35.7, 357, 7140
CSF Proteins	13-40 mg/dL	15-217 mg/dL	BSA: IgG (1:1 ratio)	Protein Concentration mg/dL 0, 30, 300, 1,071
Blood	None	Not applicable	14 bloody tap Human CSF	0% to approximately 2.5% v/v blood
Hemoglobin	12-18 g/dL RBC	Not applicable except in bloody taps	Hemoglobin (Ferrous powder) spiked into CSF	HgB g/dL 0, 0.36, 0.71, 2.14, 3.6 [Represents approximately v/v blood in CSF, respectively: 0%, 2.5%, 5%, 15%, 25%]

References

1. Viral (“Aseptic”) Meningitis. Centers for Disease Control and Prevention, National Center for Infectious Diseases: Respiratory and Enteric Viruses Branch. http://www.cdc.gov/ncidod/dvrd/revb/enterovirus/viral_meningitis.htm (accessed April 11, 2006).

2. Rotbart HA. Viral meningitis. Semin Neurol. 2000; 20(3): 277-92.

3. Romero JR, Rotbart HA. Enteroviruses. In: Murray PR, Baron EJ, eds. Manual of Clinical Microbiology. 8th edition. Washington, DC: American Society for Microbiology, 2003: 1427-1438.

4. Robinson CC, Willis M, Meagher A, et al. Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. Pediatric Infectious Disease Journal. 2002; 21: 283-6.

5. NIOSH Publication No. 2005-151: NIOSH Pocket Guide to Chemical Hazards, September 2005.

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. Wright HT, McAllister RM, Ward R. “Mixed” Meningitis: Report of a Case with Isolation of Haemophilus Influenzae Type B and ECHO Virus Type 9 from the Cerebrospinal Fluid. New England Journal of Medicine. 1962; 267: 142-144.

9. Sferra TJ, Pacini DL. Simultaneous recovery of bacteria and viral pathogens from cerebrospinal fluid. Pediatric Infectious Disease Journal. 1988; 7: 552-556.

10. Dronkert ML, Ketel AG, de Groot R. Simultaneous occurrence of group B Streptococcus and echovirus 20. European Journal of Pediatrics. 1996; 155: 915.