Assay Training: Xpert® GBS LB XC

For US-IVD Use Only

GXGBSLBXC-10
GXGBSLBXC-120

IVD In Vitro Diagnostic Medical Device

302-7973 Rev. A July 2022

Training Agenda

1. Overview
2. Kit handling
3. Sample collection
4. Preparing the cartridge
5. Quality controls
6. Result Interpretation

Training Objectives

At the end of the training, users will be able to:

- Properly store and handle the Xpert® GBS LB XC cartridge kit and sample collection
- Follow proper laboratory safety precautions
- Collect and transport appropriate specimen
- Prepare a cartridge and run the Xpert® GBS LB XC assay
- Report the various software generated results
- Understand the Xpert® GBS LB XC control strategy

Xpert® GBS LB XC

The Cepheid Solution

- Detection of Group B Streptococcus (GBS) DNA
- On-board internal controls for each sample
  - Probe Check Control (PCC)
  - Sample Processing Control (SPC)
- Closed cartridge system minimizes risk of contamination
- On-demand results
  - Results within approximately 43 minutes*
  - Early Assay Termination Positive results in as early as 27 minutes
- Random access

*With GBS negative samples, the test returns results in approximately 43 minutes following the initial 18–24 hour culture enrichment step.

Intended Use

The Xpert® GBS LB XC test, performed on the GeneXpert® Instrument Systems, is an automated qualitative in vitro diagnostic test for the detection of Group B Streptococcus (GBS) DNA from enriched vaginal/rectal swab specimens, using real-time polymerase chain reaction (PCR).

Xpert GBS LB XC testing is indicated as an aid in determining the GBS colonization status of antepartum women.

- The Xpert GBS LB XC test is intended for antepartum testing on enriched Lim broth cultures of vaginal/rectal swabs after 18–24 hours of incubation.
- The Xpert GBS LB XC test does not provide antimicrobial susceptibility test results. Culture is necessary to obtain isolates to perform susceptibility testing as recommended for penicillin-allergic women.

Targets

- The Xpert® GBS LB XC test is an automated *in vitro* diagnostic test for the qualitative detection of DNA from Group B Streptococcus (GBS).
- The primers and probes in the Xpert GBS LB XC test are designed to amplify and detect unique sequences in two GBS chromosomal targets:
  - The first is a target within a coding region for a glycosyl transferase family protein
  - The second is within a coding region for a *LysR* family transcriptional regulator of *S. agalactiae* DNA.

System and Reagent Requirements

GeneXpert® Systems

- For GeneXpert Dx System: GeneXpert Dx software **version 5.3** or higher
- For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software **version 6.8** or higher

Test Kits US-IVD

- GXGBSLBXC-10
- GXGBSLBXC-120

Sample Collection Devices

- Cepheid Collection Device (900-0370) or equivalent collection device consisting of a collection swab and transport tube with non-nutrient media.

Materials Required but not Provided

- Lim broth 5mL (Todd Hewitt broth supplemented with 15 mg/mL of nalidixic acid and 10 mg/mL colistin)
- Incubator
- Single use disposable swabs (Cepheid part number SDPS-120)
- Personal Protective Equipment (PPE)
- 1:10 dilution of bleach
- 70% ethanol or denatured ethanol

Optional

- Uninterruptable Power Supply/Surge Protector
- Printer

Good Laboratory Practice Review

Personnel Protective Equipment (PPE)

- Wear clean lab coats, wear safety glasses and gloves
- Change gloves between processing samples

Lab Bench Area

- Clean work surfaces routinely with:
  - ✓ 1:10 dilution of household bleach*
  - ✓ 70% ethanol solution
- After cleaning, ensure work surfaces are dry

Specimens, Samples, and Kits Storage

- Store specimens and samples away from kit to prevent contamination

Equipment

- Use filtered pipette tips when recommended
- Follow the manufacturer's requirements for calibration and maintenance of equipment

* Final active chlorine concentration should be 0.5% regardless of the household bleach concentration in your country.

Kit Handling

Xpert® GBS LB XC Kit Contents

Catalog Number	GXGBSLBXC-10 GXGBSLBXC-120
Cartridges* Per Kit	10 120
	Xpert GBS LB XC Assay Definition File (ADF)
Kit CD	Xpert GBS LB XC Import Instructions
	Instructions for Use (Package Insert) (PDF)
Storage	2-28 °C

* *Cartridges contain chemically hazardous substances - please see Package Insert and Safety Data Sheet for more detailed information.*

Warnings and Precautions

- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that:
  - appears wet, has leaked or if the lid seal appears to have been broken
  - appears damaged
  - has been dropped after removing it from packaging
  - has been dropped or shaken after adding the sample to it
  - has a damaged reaction tube
  - has been used; each cartridge is single-use to process one test
  - has expired
- Do not reuse pipettes/swabs/etc.

Warnings and Precautions (continued)

Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of 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.

Specimen Collection, Storage, and Transport

Specimen Collection

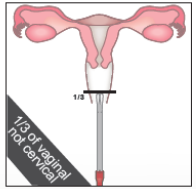
Vaginal/Rectal Specimen Collection Protocol

Important: Perform the vaginal/rectal specimen collection prior to using a speculum or using a lubricant.

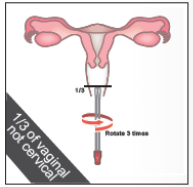
1. Use a collection device consisting of a collection swab and transport tube with non-nutrient media.

2. Use gauze to wipe away excessive secretion or discharge from the vaginal/rectal area.

3. Carefully insert the double swab into the lower third of patient's vagina and sample secretions from the mucosa.

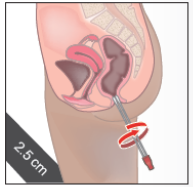


4. Rotate swab 3 times to ensure uniform sample on both swabs. Do not collect cervical sample.



5. Using the same double swab, carefully insert the swab approximately 2.5 cm beyond the anal sphincter and gently rotate to sample anal crypts.

Note: swabs must stay attached to the red cap throughout the procedure.



6. Place swabs into the tube, pushing the red cap down completely.

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Xpert® GBS LB XC Specimen Enrichment

- Follow CDC recommendations for Lim broth enrichment
- Place swab in Lim broth and incubate for 18-24 hours at 35-37 °C
- Swab specimens may be stored at room temperature for up to 24 hours before Lim broth enrichment
- Enriched Lim broth is stable at 2-8 °C for up to 72 hours following enrichment

Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep 2010, 59: 1-36

Xpert® GBS LB XC Specimen Transport and Storage

- Pre-Enrichment

Specimen Type	Storage
Vaginal/rectal swab	2-8ºC up to 2 days 15-30ºC up to 24 hours

- Post-Enrichment

Specimen Type	Storage
Enriched Lim broth	2-8ºC for 3 days

Preparing the Cartridge

Xpert® GBS LB XC Cartridge Preparation

Xpert® GBS LB XC Cartridge Preparation

Refer to the package insert for detailed instructions, precautions, and warnings.

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

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Cepheid Part Number SDPS-120

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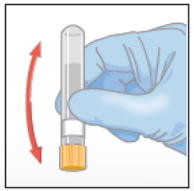
A better way.

1 Obtain one cartridge.

2 Open the cartridge lid.

3 Mix the Lim broth tube by vigorous shaking or vortexing for 5 seconds.



4 Dip the clean single use disposable swab into the Lim broth.

5 Insert the swab into the cartridge. Break the swab at the score mark.

Make sure the swab can float freely in the chamber.

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

6 Close the cartridge and start the test within the timeframe specified in the package insert.

Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.

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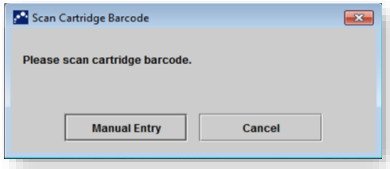
Run a Test on GeneXpert® Dx

1 Create a test.

Start the test within 30 minutes after adding the sample to the cartridge.

2 Scan barcode for Patient and/or Sample ID.



Do not click on Manual Entry or Cancel.

3 Scan the cartridge.

For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx Operator Manual.

Run a Test on GeneXpert® Dx (continued)

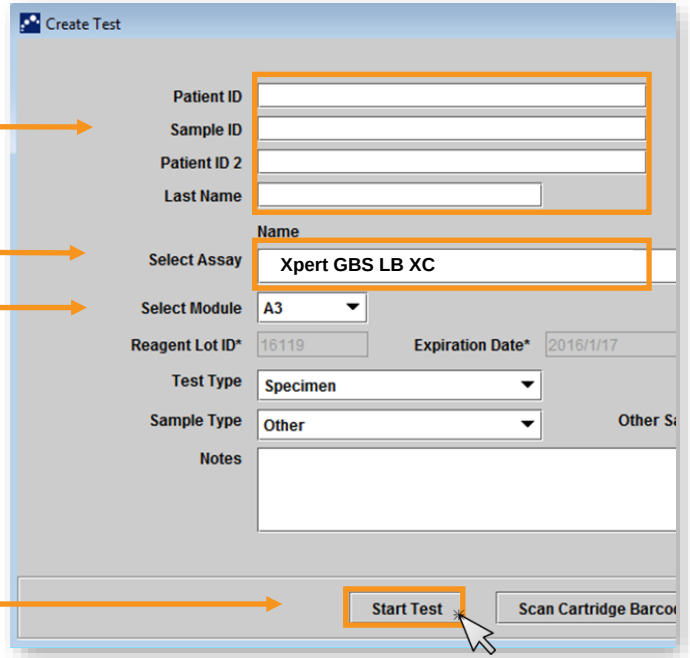
4 Complete the fields as required.

5 Xpert® GBS LB XC test is selected automatically.

6 The module is selected automatically.

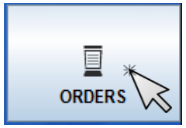
7 Click on Start Test.

8 A green light will flash on the module.
Load the cartridge into module and close the door.



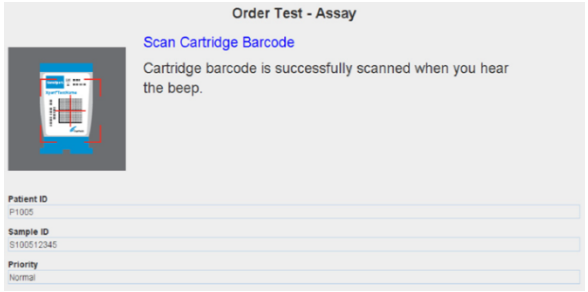
Run a Test on GeneXpert® Infinity

1 Create a test.

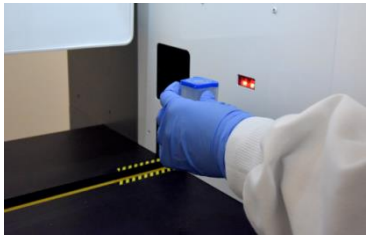


Place the cartridge on the conveyor within 30 minutes of adding the sample.

2 Scan barcode for Patient and/or Sample ID.



3 Scan the cartridge.



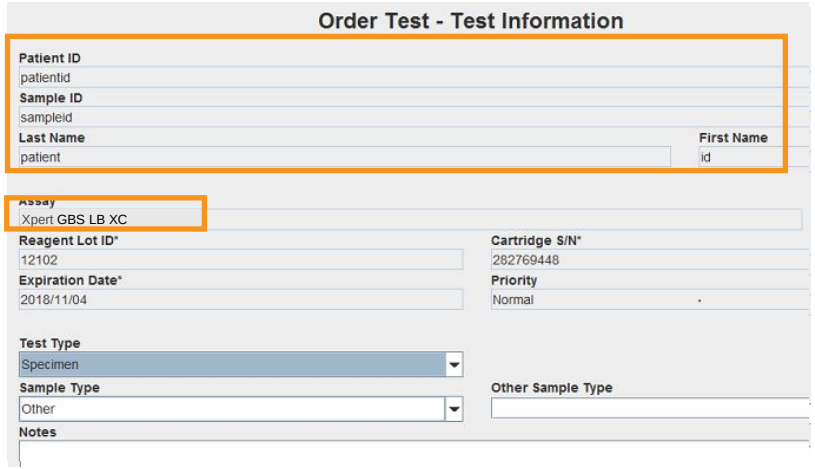
For complete details on how to run a test, refer to the Package Insert and the GeneXpert Infinity Operator Manual.

Run a Test on GeneXpert® Infinity (continued)

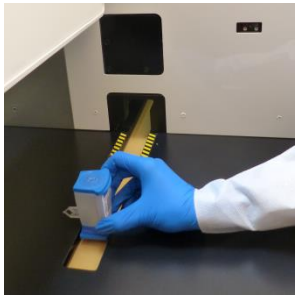
4 Complete the fields as required.

5 Xpert® GBS LB XC is selected automatically.

6 Click SUBMIT.



7 Place the cartridge onto the conveyor belt.



Automated Xpert® GBS LB XC Protocol

1. Sample is added to the cartridge
2. The cartridge is loaded into the System
3. Nucleic acids are purified
4. Purified nucleic acids mix with the PCR reagents
5. Simultaneous amplification and detection occurs
6. Results are ready to view

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Quality Controls

Cepheid Assay Control Strategy

- Each Xpert cartridge is a self-contained test device.
- Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
  - Probe Check Control (PCC)
  - Sample Processing Control (SPC)

Internal Quality Controls

- **Probe Check Controls (PCC)**
  - Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor

    bead rehydration
    probe integrity
    reaction tube filling
    dye stability

- **Sample Processing Controls (SPC)**
  - Verifies adequate sample processing
  - Verifies lysis, presence of the organism and detects PCR inhibition
  - Should be positive in a negative sample
  - Can be positive or negative in a positive sample

Commercially Available External Controls

Company	Description	Catalog Number
ZeptoMetrix®	*Streptococcus* species (Group B) positive control	NATSAG-6MC
ZeptoMetrix®	*L. acidophilus* as a negative control	NATLAC-6MC

External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

Results Analysis

Results Summary

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

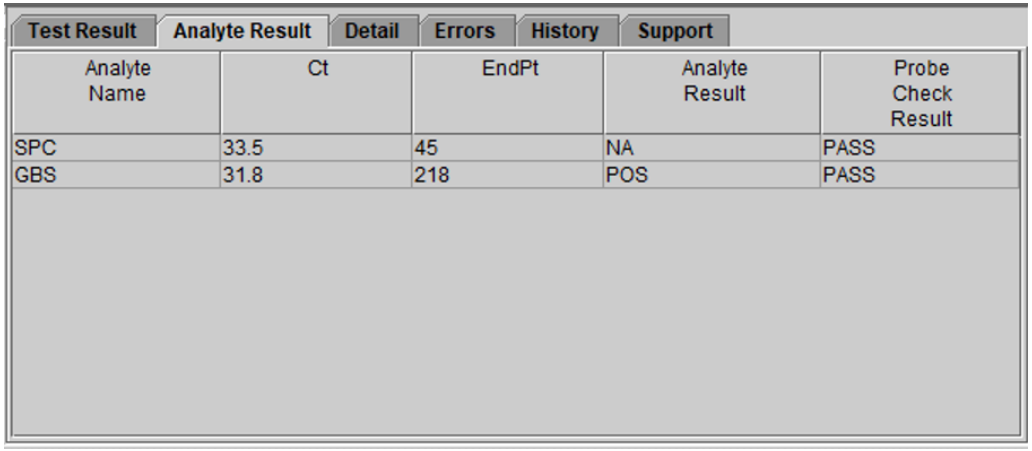
EAT (Early Assay Termination)

- **What is it?**
  - Real-time monitoring of reaction progress
  - Termination of the reaction when the cycle threshold of a particular reaction is crossed

- **What are the benefits?**
  - Positive results are reported sooner (dependent on sample titer)
  - For time-critical interventions, valuable minutes are saved for patients that need it the most

GBS Positive

GBS POSITIVE



Test Result | Analyte Result | Detail | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	33.5	45	NA	PASS
GBS	31.8	218	POS	PASS

GBS target nucleic acid is detected.

- SPC: N/A
  The SPC is ignored because GBS amplification can compete with control.

- Probe Check: PASS
  All probe check results pass.

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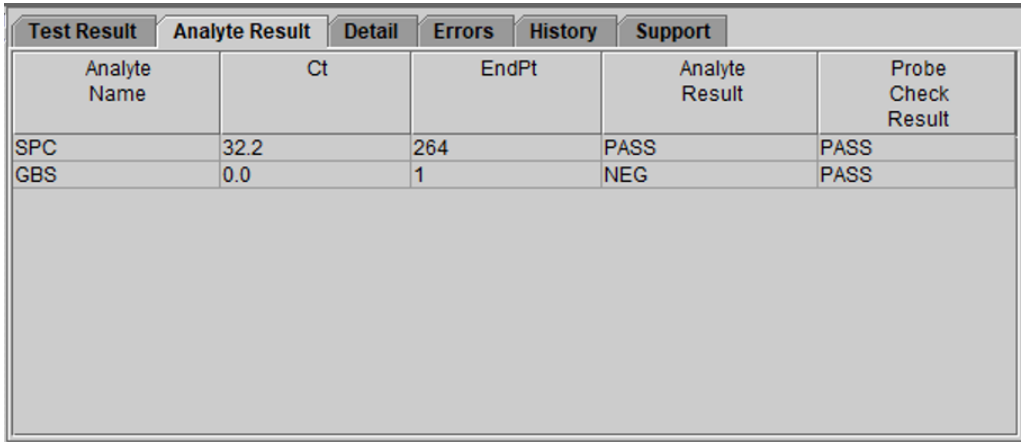
GBS Negative

GBS NEGATIVE



Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	32.2	264	PASS	PASS
GBS	0.0	1	NEG	PASS

GBS target nucleic acid is not detected.

- GBS: NEG
- SPC: PASS
  The SPC has valid Cts.
- Probe Check: PASS
  All probe check results pass.

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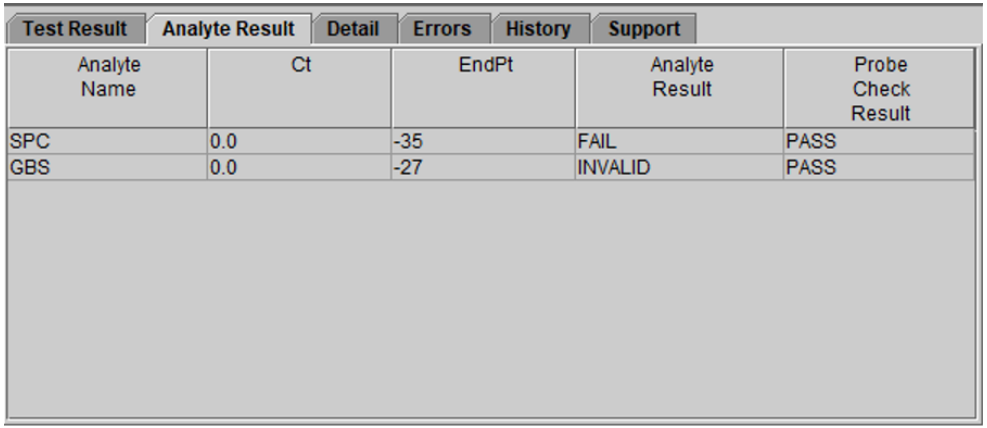
Troubleshooting

Factors That Negatively Affect Results

- **Improper specimen collection**
  - Performance with other collection devices and specimen types has not been assessed.
- **Improper transport or storage of collected specimen**
  - Storage and transport conditions are specimen specific.
  - Refer to the Package Insert for the appropriate handling instructions.
- **Improper testing procedure**
  - Modification to the testing procedures may alter the performance of the test.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the Package Insert is necessary to avoid erroneous results.
- **Interfering substance**
  - False negative test results or invalid results may be observed in the presence of interfering substances.
- **The number of organisms in the specimen is below the detection limit of the test**
- **Mutations in primer or probe binding regions may affect detection of new or unknown GBS variants resulting in a false negative result.**

INVALID

INVALID

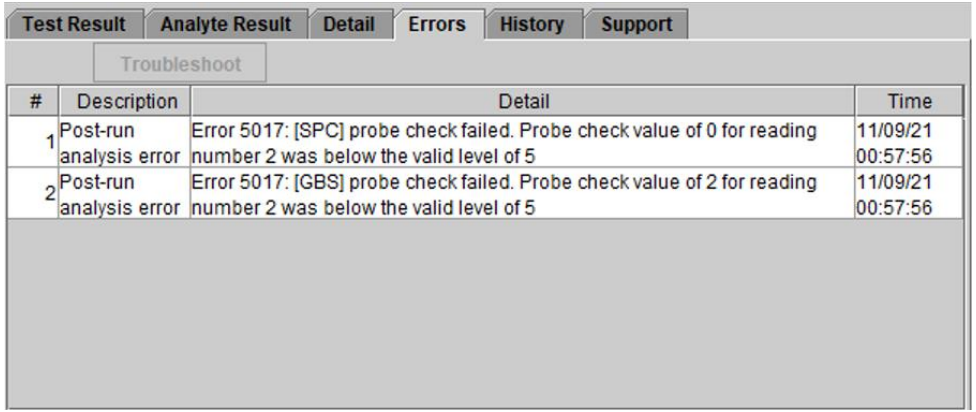


Presence or absence of GBS target DNA cannot be determined. SPC does not meet acceptance criteria.

- GBS — INVALID
- SPC — FAIL
- Probe Check Controls—PASS

ERROR

ERROR

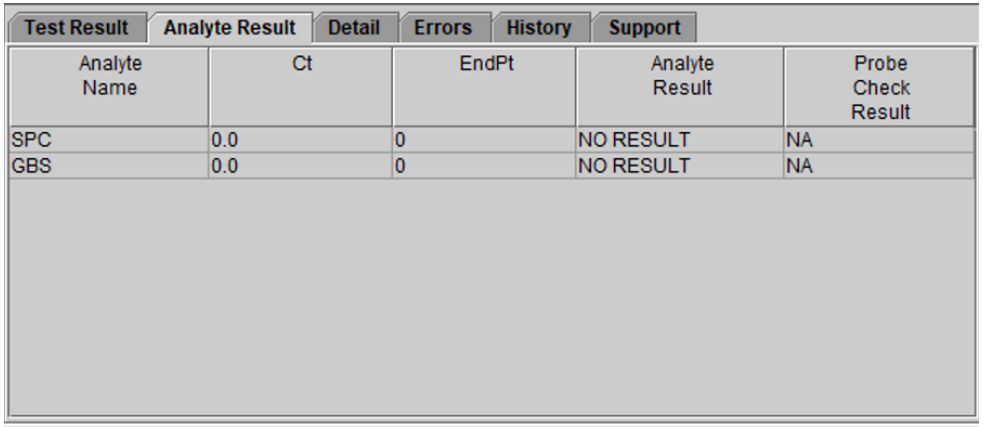


Presence or absence of GBS target DNA cannot be determined. A system component failed, the maximum pressure was reached, or the probe check failed.

- GBS — NO RESULT
- SPC — NO RESULT
- Probe Check Controls—FAIL

NO RESULT

NO RESULT



Test Result | Analyte Result | Detail | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	0.0	0	NO RESULT	NA
GBS	0.0	0	NO RESULT	NA

Presence or absence of GBS target DNA cannot be determined. Insufficient data were collected. For example: the operator stopped the test, or a power failure occurred during the test.

- GBS — NO RESULT
- SPC — NO RESULT
- Probe Check Controls—NA (not applicable)

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Xpert® GBS LB XC Retest Procedure

1

Discard used cartridge

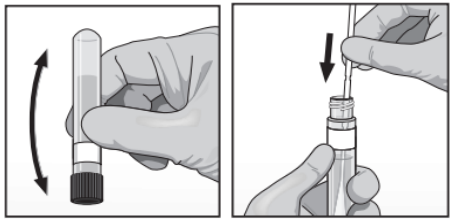
Follow your institution's safety guidelines for disposal of cartridges.

2

Obtain a new cartridge.

Process the sample per the package insert.

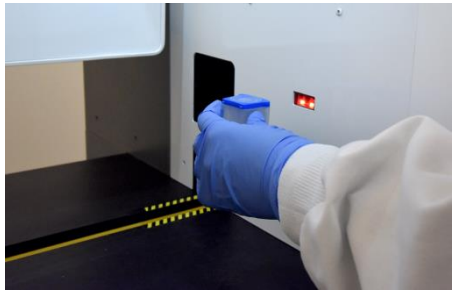
3



- Obtain the remaining enriched Lim broth.
- Invert the Lim broth tube 3 times to mix.
- Insert a new single use disposable swab into the Lim broth.
- Insert the swab into the S chamber of a new Xpert GBS LB cartridge and re-run the test.

4

Run the test on the system.



Limitations

- Erroneous test results might occur from improper specimen collection, handling or storage, technical error, or sample mix-up. Careful compliance to the instructions in this insert is important to avoid erroneous results.
- A negative result does not rule out the possibility of GBS colonization. False negative results may occur if the organism is present at levels below the analytical limit of detection.
- The performance of the Xpert® GBS LB XC test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- The Xpert GBS LB XC test has been validated with Lim broth medium only. Performance of the assay has not been validated with other GBS selective broth enrichment media.

Limitations (continued)

- Culture isolates are needed for performing antibiotic susceptibility testing as recommended for penicillin-allergic women. Use remaining enriched Lim broth to obtain culture isolates. Laboratories must validate their own culture procedures.
- Good laboratory practices should be followed.
- Culture test results may be affected by concurrent antibiotic therapy. GBS DNA may continue to be detected following antimicrobial therapy.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- A positive result does not necessarily indicate the presence of viable organisms.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown variants and may result in a false negative result.

Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
  - Product name
  - Lot number
  - Serial number of the System
  - Error messages (if any)
  - Software version

- Log your complaint online using the following link ***http://www.cepheid.com/en/support:*** *Create a Support Case*

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A better way.

Thank You

www.Cepheid.com