PROCEDURE: Xpert® GBS LB XC

I. PRINCIPLE

Group B *Streptococcus* (GBS) bacterial infection is associated with serious illness in infants born to women who are colonized with *Streptococcus agalactiae*. Transmission occurs from GBS-colonized women to their newborn antepartum or during birth. Infants with GBS infection can develop sepsis, pneumonia, or meningitis. CDC guidelines recommend the use of nucleic acid amplification testing (NAAT) in addition to traditional culture as an aid in determining GBS colonization status in antepartum women. The Xpert GBS LB XC Assay is a rapid PCR test for the detection of Group B streptococci from enriched vaginal/rectal swab specimens. This platform automates and integrates sample preparation, nucleic acid extraction, amplification, and detection of the target sequences in clinical specimens using real-time PCR. The assay is performed on Cepheid GeneXpert Instrument systems. The Xpert GBS LB XC 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 system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR processes. Because the cartridge is self-contained, the risk of cross-contamination between samples is minimized. The Xpert GBS LB XC has an Early Assay Termination (EAT) function that enables early result reporting. EAT is activated when the pre-determined threshold for a positive test result is reached before the full number of PCR cycles have been completed. EAT can reduce the test time for positive results as early as 27 minutes. With GBS negative samples, the test returns results in approximately 43 minutes following the initial 18–24-hour enrichment step.

II. AVAILABILITY

A. Batched testing performed once daily on specimens received before 11am the previous day.

III. TEST CODE

A. GBSPC

IV. SPECIMEN COLLECTION AND TRANSPORT

A. Vaginal/Rectal swab specimen collected on a Copan Dacron swab or eSwab®.
B. Swab specimens may be stored at room temperature for up to 24 hours, or at 2-8°C for up to 48 hours before processing in Lim broth for enrichment.
C. Enriched Lim broth is stable at 2-8°C for up to 72 hours.

V. MATERIALS AND EQUIPMENT

A. Materials
    1. Xpert GBS LB XC Assay kits (GXGBSLBXC-10 or GXGBSLBXC-120)
    2. Single Use, Disposable swab for processing Lim broth specimens (Cepheid SDPS-120)
    3. LIM Broth 5mL (inoculated)
B. Equipment
    1. Biosafety Cabinet
    2. Vortex Genie
    3. GeneXpert Infinity System
    4. Xpertise software version 6.8 or higher

VI. STORAGE AND HANDLING

A. Store the Xpert GBS LB XC Assay cartridges at 2-28 °C.
B. Do not use cartridges that have passed the expiration date on the label.

C. Do not use a cartridge that has leaked.
D. Do not open the cartridge lid until you are ready to perform testing.

VII. QUALITY CONTROL

A. Maintenance
    1. Cleaning and maintenance of the instrument will be performed in accordance with the manufacturer's Operator's Manual. For further information, refer to the Infinity System's Operator's Manual.
B. External Controls
    1. Run every 30 days or upon receipt of new lots/shipments of kits, after system repairs, and software upgrades.
    2. Positive and Negative external controls routinely obtained from Zeptometrix or designated distributor. Previously analyzed known patient positive or negative specimens may be substituted if commercial controls are unavailable.
        a. Positive – NATSAg-MC (*S. agalactiae*)
        b. Negative – NATLAc-MC (*L. acidophilus*)
            1) Control Preparation
                a) Vortex control sample for 5 seconds.
                b) Open the cartridge lid.
                c) Inoculate a clean sterile swab, place the swab into the cartridge and snap the shaft by pulling it to the right.
                d) Close the cartridge lid.
                e) Run cartridge as usual.
C. Internal Controls
    1. Sample Processing Control (SPC): Ensures the sample was correctly processed by monitoring the lysis and elution processing. SPC should pass – generate a valid cycle threshold (Ct) in a negative sample – but may not amplify in a high-positive sample.
    2. Probe Check Control (PCC): 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.
D. Environmental Contamination Checks
    1. Wipe testing performed every 30 days or as needed to rule out potential contamination.
E. Refer to the Individualized Quality Control Plan (IQCP) for the Genexpert GBS LB XC assay for complete details of the QC data and QA plan approved by the laboratory Director.

VIII. TEST PROCEDURE

A. Pre-analytical – Batched processed at 11am daily
    1. Label Lim broth with appropriate specimen label.
    2. Remove swab from the transport collection tube and place it into the Lim broth.
    3. Break the swab off leaving it in the Lim broth.
    4. Place the inoculated Lim broth into the designated rack in 35-37 °C ambient incubator for overnight incubation.
B. Adding sample to the cartridge
    *****Gloves should be changed between handling of each individual patient specimen**
    1. Remove the cartridge from the box and open the lid.
    2. Mix the Lim broth by vortexing for 5 seconds.
    3. Dip a clean single use, disposable swab into the LIM broth.
    4. Transfer the swab into the Xpert GBS LB XC cartridge sample chamber.
    5. Raise the swab so that the score mark is centered in the notch.
    6. Break the swab by snapping the shaft at the score line.
    7. Ensure the swab is properly positioned in the cartridge and the swab end is not in the notch of the sample chamber prior to closing the cartridge.
    8. Close the cartridge and load it onto the GeneXpert instrument.

C. Starting the test
1. Log into Windows operating system (if not already open).
    a. Enter the designated Cepheid PC log in.
2. Log on to the System software.
    a. Use your individual login information for Xpertise software.
3. In the GeneXpert System window, click **Orders → Order Test**.
4. Scan (or type) in the Patient ID and scan the barcode on the Xpert GBS LB XC Assay cartridge.
5. Click **Submit**.
6. After clicking Submit, you will be asked to place the cartridge on the conveyor belt.
7. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the rest will run, and the used cartridge will be placed onto the waste shelf for disposal.

IX. INTERPRETATION

A. The results are interpreted automatically by the GeneXpert Instrument System and are shown in the View Results window. The possible results and interpretations are show below:

Result	Interpretation
GBS NEGATIVE	GBS Target DNA is not detected • GBS – NEG • SPC – PASS • Probe Check Controls - PASS
GBS POSITIVE	GBS Target DNA Detected • GBS – POSITIVE • SPC – NA (not applicable) • 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 – NOT RESULT • Probe Check Controls – **FAIL **If the probe check passed, the error is caused by a system component failure or by exceeding maximum allowable pressure.
NO RESULT*	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)

*If an INVALID, ERROR, or NO RESULT occurs, repeating according to Retest Procedure below.

B. Retest Procedure
<u>Note</u>: Performed on tests that result as INVALID, NO RESULT, or ERROR

1. Mix the leftover patient specimen by vortexing for 5 seconds.
2. Insert a single use disposable swab into the Lim broth.
3. Insert the swab into the Sample chamber of the Xpert GBS LB XC cartridge and rerun the test.
4. If the re-testing is not successful, result the GBSPC order as Indeterminate and reflex the order to a CXGRB.
5. Inoculate a BAP using the incubated LIM broth and incubate overnight.

X. RESULTING

A. Refer to Appendix AP64 – Soft instructions for Cepheid Genexpert for complete instructions.
B. Specimens are resulted as either DETECTED, Not Detected, or Invalid with pre-populated comments when required.

XI. ADDITIONAL TESTING

A. LIM broths will be saved for 7 days in case a susceptibility is requested.
B. When a susceptibility is requested, order a **CXGRB** and sub the LIM broth to a BAP and incubate overnight.
C. After overnight incubation, confirm that Group B Streptococcus is present using the PathoDx Strep Grouping kit.
NOTE: Isolation may be necessary before testing due to growth of vaginal/rectal flora
D. After confirming the presence of Group B Streptococcus, set up a Kirby-Bauer susceptibility. Refer to Procedure: Group B Streptococcus screening for required antibiotics.
E. If Group B Strep is not isolated in the culture, result the order with the comment:

"Susceptibilities unable to be performed. Organism is non-viable."

F. Make a notification call to the requesting provider and document that the call was made in the order.

XII. LIMITATIONS OF TEST

A. Erroneous test results can occur due to improper specimen collection, technical error, sample mix-up, or because of a low number of organisms in the specimen. Swabbing both the lower vagina and rectum increases the yield compared to sampling the cervix or vagina without also swabbing the rectum.
B. A negative result does not rule out the possibility of GBS colonization. False negative results may occur when the GBS concentration in the specimen is below the limit of detection.
C. The performance of the assay was evaluated using the procedures provided in the package insert only. Modifications to these procedures may alter the test performance.
D. This assay has been validated with Lim broth medium only.
E. Mutations in primer or probe binding regions may affect detection of new or unknown GBS variants and may result in a false negative result.
F. Positive results do not definitively indicate the presence of viable organisms.
G. Patients who have used topical or systemic antibiotic treatment in the previous week as well. as patients diagnosed with placenta previa should not be tested.
H. A positive result does not necessarily indicate the presence of viable organisms.

XIII. TECHNICAL SUPPORT

A. (888) 838-3222
B. techsupport@cepheid.com

XIV. ATTACHMENTS

A. Appendix AP64 – Soft instructions for Cepheid GeneXpert

XV. REFERENCES

A. Xpert® GBS LB XC Package Insert 302-4580, Rev. B October 2022