**PROCEDURE: GROUP B STREP BY PCR**

1. **PRINCIPLE**

Group B streptococci (GBS) are gram-positive, beta-hemolytic bacterial pathogens associated with serious illness in newborns born to women who are colonized with the organism. Transmission occurs from GBS-colonized women to their newborn antepartum or during birth. It is a major cause of death in newborns who 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 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 with fluorogenic detection of the amplified DNA. The assay is performed on Cepheid GeneXpert Instrument systems. The Xpert GBS LB 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 time to result is approximately 55 minutes after the initial 18-24 hour enrichment step.

1. **AVAILABILITY**
   1. 24/7 AT RIH, TMH & NH
   2. Specimens received for testing will be batch processed every morning.
2. **TEST CODE**
   1. GBSPC
3. **SPECIMEN**
   1. Vaginal/Rectal
      1. Collected on a Copan Double Dacron swab or ESWAB®.
4. **MATERIALS AND EQUIPMENT**
   1. Materials
      1. Swabs
         1. Single Use, Disposable, Sterile
      2. Xpert GBS LB Assay kits
      3. LIM Broth (inoculated)
         1. 5mL
      4. Quality Control Material
         1. NATtrol Strep B positive control (NATSAg-MC)
         2. NATtrol Strep B negative control (NATLAc-MC)
   2. Equipment
      1. Biosafety Cabinet
      2. GeneXpert Infinity System
      3. Xpertise software version 6.4b or higher
5. **STORAGE AND HANDLING**
   1. Store the Xpert GBS LB Assay cartridges at 2-28 until the expiration date

provided on the label.

* 1. Do not open the cartridge lid until you are ready to perform testing.
  2. Do not use cartridges that have passed the expiration date.
  3. Do not use cartridges that have leaked
  4. Start the test within 15 minutes of adding the sample to the cartridge

1. **QUALITY CONTROL**
   1. Maintenance
      1. Cleaning and maintenance of the instrument will be

performed in accordance with the vendors Operator’s Manual. For further information, refer to the Infinity System’s Operator’s Manual

* 1. External Controls
     1. Positive – NATSAg-MC (S. agalactiae)
     2. Negative – NATLAc-MC (L. acidophilus)
     3. Control Preperation
        1. Vortex NATrol sample for 5 seconds.
        2. Open the cartridge lid.
        3. Inoculate a clean sterile swab, place the swab into the

cartridge and snap the shaft by pulling it to the right.

* + - 1. Close to cartridge lid.
  1. Internal Controls
     1. Sample Processing Control (SPC)
        1. Detect correct processing of sample
        2. Detection of sample-associated inhibition
        3. Ensures appropriate reaction conditions
     2. Probe Check Control
        1. Check of fluorescence signal
        2. Monitors bead rehydration, reaction tube filling, probe

integrity & dye stability.

* 1. Environmental Contamination Checks
     1. Performed Every 30 Days
  2. Expected Results

|  |  |
| --- | --- |
| **Control** | **Expected Results** |
| External Positive | POSITIVE – target DETECTED |
| External Negative | Negative – no target detected |
| SPC | POSITIVE in a negative patient sample  Negative or POSITIVE in a POSITIVE patient sample |
| PCC | Thresholds are preprogrammed by manufacturer |
| Environmental Check | Negative – no target detected |

1. **TEST PROCEDURE**
   1. Pre-analytical (performed when specimens are received in the lab)
      1. Label Lim broth with appropriate specimen label.
      2. Remove swab from the transport collection tube and place it into

the Lim broth.

* + 1. Break the swab off leaving it in the Lim broth.
    2. Place the inoculated Lim broth into the rack for overnight

incubation.

* 1. Adding sample to the cartridge
     1. Remove the cartridge from the box and open the lid.
     2. Invert the enriched LIM broth tube three times to sufficiently mix the

specimen.

* + 1. Dip a clean single use disposable swab into the LIM broth.
    2. Transfer the swab into sample(S) chamber of the Xpert GBS LB

cartridge.



* + 1. Raise the swab so that approximately 1” of it is still within the

sample chamber.

* + 1. Break the swab by snapping the shaft to the right.
    2. Close the cartridge and load it onto the GeneXpert instrument.
  1. Starting the test
     1. Log into Windows operating system (if not open).
        1. You will need to use your SOFT login and password.
     2. Log on to the System software
        1. You will use your individual login information for the Cepheid
     3. In the GeneXpert System window, click **Orders** followed by

**Order Test**.

* + 1. Scan (or type) in the patient ID, Sample ID and Scan the barcode

on the Xpert GBS LB Assay cartridge.

*[NOTES: If typing, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result. Using the barcode information; the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date, and Selected Assay.]*

* + 1. Click **Submit** if Auto-submit is not enabled.
    2. After clicking Submit, you will be asked to place the cartridge

on the conveyor belt. 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.

* 1. Retest Procedure

Note: Repeat testing will be performed on tests that result as INVALID, NO RESULT, or ERROR.

* + 1. Mix the leftover patient specimen by inverting 3 times.
    2. Insert a single use disposable swab into the Lim broth.
    3. Insert the swab into the S chamber of the Xpert GBS LB cartridge

and rerun the rest.

* + 1. If the re-testing is not successful, result the GBSPC order as Indeterminate and reflex the order to a CXGRB.
    2. Inoculate a BAP using the incubated LIM broth and incubate overnight.

1. **INTERPRETATION**
   1. 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 Nucleic Acid Is Not Detected  SPC – PASS  IC – PASS  Probe Check - PASS |
| **GBS POSITIVE\*** | GBS Target Nucleic Acid Is Detected  SPC – NOT APPLICABLE  IC – NOT APPLICABLE  Probe Check - PASS |
| **INVALID\*** | GBS Target Nucleic Acid Presence or Absence Cannot Be Determine  SPC – FAIL  IC – FAIL  Probe Check - PASS |
| **ERROR\*** | Presence or Absence of GBS Target Nucleic Acid Cannot Be Determined.  (System component failed, maximum pressure was reached, or probe check failed)  GBS – NO RESULT  SPC – NOT RESULT  IC – NO RESULT  Probe Check – Fail (If PC Passed, error caused by system component failure) |
| **NO RESULT\*** | Presence or Absence of GBS Target Nucleic Acid Cannot Be Determined.  (Operator stopped the test, a power failure occurred, or problems were detected in the cartridge)  GBS – NO RESULT  SPC – NO RESULT  IC – NO RESULT  Probe Check: Not Applicable |

\*Refer to example graphs in the Package Insert for more information.

1. **ADDITIONAL TESTING**
   1. LIM broths will be saved for 7 days, in case a susceptibility is needed.
   2. When a susceptibility is requested, order a “CXGRB” and sub the LIM

broth to a BAP and incubate overnight.

* 1. 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

* 1. After confirming the presence of Group B Streptococcus, set up a Kirby-Bauer susceptibility. Refer to *Procedure: GROUP B STREPTOCOCCUS SCREENING*for required antibiotics.
  2. 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”. Make a notification call to the requesting provider and document that the call was made in the order.

1. **LIMITATIONS OF TEST**
   1. 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.
   2. 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.
   3. This assay has been validated with Lim broth medium only.
   4. Mutations in primer or probe binding regions may affect detection of new or unknown GBS variants results in a false negative result.
   5. Positive results do not definitively indicate the presence of viable organisms.
   6. 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.
2. **TECHNICAL SUPPORT**
   1. 1(888) 838-3222 or [techsupport@cepheid.com](mailto:techsupport@cepheid.com)
   2. Before contacting Cepheid Technical Support, collect the following

information:

* + 1. Product name
    2. Lot number
    3. Serial number of the instrument
    4. Error messages (if any)
    5. Software version and (if applicable) Computer Service Tag number
  1. Restarting the System:
     1. Make sure instrument is not processing samples
     2. Remove all cartridges from modules
     3. Quit GeneXpert Software
     4. Turn off the computer
     5. Turn off the instrument
     6. Wait 2 minutes
     7. Turn on the instrument
     8. Wait 2 minutes
     9. Turn on the GeneXpert Software.

1. **REFERENCES** 
   1. Xpert® GBS LB Package Insert. Document: 301-0576, Rev. B August 2014