UPMC HANOVER LABORATORY

Subject: Cepheid MRSA NxG	

PURPOSE:

The MRSA NxG Assay is a qualitative in vitro diagnostic test intended for the detection of methiciliin-resistant Staphylococcus aureus (MRSA.) DNA directly from nasal swabs in patients at risk for nasal colonization. It is intended to aid in the prevention and control of MRSA infections in the healthcare setting. The Xpert MRSA NxG Assay is not intended to diagnose, guide, or monitor treatment for MRSA infections, or provide resultls of susceptibility to methicillin. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

The Xpert MRSA NxG test is performed on the GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequence in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require 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 minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.*

The primers and probes in the Xpert MRSA NxG test detect proprietary sequences for methicillin/oxacillin resistance (*mecA* and *mecC* genes), and SCC*mec*, which is inserted into the SA chromosome at the *attB* site. An Early Assay Termination function provides positive results if target DNA reaches a predetermined threshold before the full 40 PCR cycles have been completed. When MRSA target levels (*mecA/mecC* and SCC*mec*) are high enough to generate very early Cts, the SPC amplification curve will be not seen, and its results will not be reported.

SCOPE:

This policy applies to all UPMC Hanover Hospital laboratory.staff trained in use of Cepheid MRSA PCR Testing.

SPECIMEN:

Swabs collected from both nares, such as the swabs supplied in the Cepheid Sample Collection Device (Dual rayon swab in Liquid Stuarts medium), Copan Dual Rayon Swab and Transport Systems, Liquid Amies Elution Swab (ESwab) Collection and Transport System or BD ESwab Collection Kit.

Specimen Stability: 24 hours at 15-30 C.
7 days at 2-8 C.

MATERIALS:

1. 10% Clorox
2. 70% Ethanol
3. Vortex Mixer
4. Sterile Transfer Pipette

5. Gauze or Kimwipes
6. Biosafety Hood
7. Micropipette capable of measuring 100ul and 300ul
8. MRSA NxG cartridge kit
    a. MRSA NxG cartridge
    b. Elution Reagent vial

Notes:

- Store kits at 2-28C.
- Do not use kits that have passed the expiration date.
- Do not open a cartridge lid until you are ready to perform testing.
- Elution Reagent is a colorless liquid. Do not use if it has become discolored.

9. NATrol MRSA Positive and Negative Quality Controls, Zeptometrix Corporation.

HAZARDS/PRECAUTIONS:

1. All specimens should be considered biohazardous and should be handled as per hospital policy.
2. Dispose of used cartridges immediately after processing.

RECORDS/FORMS/DOCUMENTS:

1. Cepheid MRSA NxG Kit QC

CALIBRATION: N/A

QUALITY CONTROL:

1. **External Controls Frequency:**

IQCP will be put in place after 30 consecutive testing days of QC with no failures.
External Controls are run at the following intervals (IQCP plan in place):

- When new shipment/lot is received
- At least every 31 days
- If major instrument maintenance is performed.
- If major software update is installed

External QC:

NATrol MRSA positive and negative control, Zeptometrix Corporation

(a) Select the appropriate positive and negative QC vials.

(b) Vortex the vial for ten seconds to ensure thorough mixing.

(c) Using a filtered pipette tip, pipette 100ul of the QC sample to the vial containing sample reagent. Vortex the reagent vial for 5-10 seconds.

(d) Open the cartridge lid. Using a clean transfer pipette (not supplied), transfer the entire contents of the Sample Reagent to the Sample Chamber of the Xpert MRSA NxG cartridge.

(e) Proceed with "Starting the test" below, typing Pos/Neg Lot Shipment QC/30 Day QC as appropriate for Sample ID.

Record all QC results on LABM011 in the Rapid Tests QC Notebook.

If the expected QC results are not obtained, do not report patient test results.

Repeat QC testing, and if still unacceptable, notify supervisor and call Cepheid technical support.

2. **Internal Controls**

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

a) **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.

b) **Probe Check Control (PCC)** - Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability.

Valid results are not given if Internal Controls Fail

PROCEDURE:

NOTE: All manipulations of culture specimens must be performed in the BSC (biosafety cabinet).

- Use 10% Bleach followed by 70% Ethanol for cleaning Biosafety Hood and to clean workspace under the hood:
  - Prior to beginning specimen processing
  - If there are any spills (**Hood only needs to be cleaned between patients, if there was spillage of specimen in hood**)
  - After all processing is complete.
- Change gloves prior to handling any patient specimens AND between each patient.
- ONLY have 1 Patient Sample, 1 cartridge, 1 sterile swab, 1 piece of gauze under hood at a time.
- When opening any specimen or control in hood, place cap with contaminated side up to help prevent any contamination of work area and replace lid prior to changing gloves.
- Do not use a cartridge that has been dropped after removing it from the packaging. Dropping may yield invalid results.
- Do not shake the cartridge. Shaking may yield invalid results.
- Do not reuse spent cartridges.
- Do not use a cartridge that has a damaged reaction tube.

Preparing the Cartridge:

1. *Preparing the Cartridge*

   Place the cartridge into the GeneXpert instrument within 30 minutes of adding the Elution Reagent to the cartridge.

   a. Remove a cartridge and Elution Reagent vial from the Xpert MRSA NxG test.
   b. Mix the eSwab contents by vortexing for 5 seconds at high speed to release the sample from the swab tip.
   c. Transfer 300 µl of the liquid sample into the Elution reagent vial.
   d. Close the Elution Reagent vial cap and vortex at high speed for 10 seconds.
   e. Open the cartridge lid. Using a transfer pipette transfer the entire contents of the elution Reagent vial to the Sample Chamber of the Xpert MRSA NxG test cartridge. Close the cartridge lid and proceed to Starting the Test.

Figure 1. Xpert GBS LB XC Cartridge (Top View)

2. **Starting the Test**

   Always remove or change gloves prior to loading cartridges into instrument.

a) Log into instrument by entering login and password.
b) Click on **Create Test**
c) Scan Patient Barcode or manually enter Accession Number (in the **Sample ID** box)
d) Scan the barcode on the Cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: **Select Assay, Reagent Lot ID**, **Cartridge S/N and Expiration Date**
e) If Accession Number was manually entered, enter the patient's Medical Record Number (in the **Patient ID** box).
f) Click on **Start Test** (if dialogue box appears, enter your username/password)
g) Open the instrument module door, with the blinking green light, and load the cartridge. DON'T touch the reaction tube (the part that extends out of the back of the cartridge)
h) Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
i) Wait until the system releases the door lock before trying to open the module door and removing the cartridge.
j) Dispose the used cartridge in biological waste (red bag) trash.

- **NOTE: DO NOT CLOSE (exit) OUT OF THE GENEXPERT WHEN RUNNING. IF YOU DO, ANY TESTING IN PROGRESS WILL ABORT!!!**

- **ALWAYS dispose of used cartridges when testing is complete and do not allow them to sit in the module. This can lead to contamination of the system.**

- Keeps doors to the Cepheid Modules closed (not tightly) when not in use.

INTERPRETATION OF RESULTS AND REPORTING:

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

a. Click the **View Results** icon to view results.
b. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

The results are interpreted by the GeneXpert System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.

Table 2. Xpert MRSA NxG Test Results and Interpretation

Result	Interpretation
MRSA DETECTED	• MRSA DETECTED: MRSA targets, *mec* (*mecA/mecC*) and SCC*mec*, have a cycle threshold (Ct) within the valid range. • SPC – NA (not applicable); the SPC signal is not part of the results interpretation algorithm if MRSA is detected since SPC signal may be suppressed due to competition with *mec* (*mecA/mecC*) and SCC*mec*. • Probe Check – PASS; all probe check results pass.
MRSA NOT DETECTED	• MRSA NOT DETECTED: Scenarios • Target DNA for SCC*mec* is not detected and target DNA for *mec* (*mecA/mecC*) is not detected • Target DNA for SCC*mec* is not detected and target DNA for *mec* (*mecA/mecC*) is detected • Target DNA for SCC*mec* is detected and target DNA for *mec* (*mecA/mecC*) is not detected • SPC: PASS; SPC has a Ct within the valid range and both target DNA *mec* (*mecA/mecC*) and SCC*mec* are not detected. Or, if either the *mec* (*mecA/mecC*) or SCC*mec* exhibit a valid Ct value, SPC result is ignored. • Probe Check — PASS; all probe check results pass.
INVALID	Presence or absence of MRSA target DNA (*mecA/mecC* or SCC*mec*) cannot be determined. Repeat the test. • Target DNA for SCC*mec* is not detected and target DNA for *mec* (*mecA/mecC*) is not detected. • SPC: FAIL; SPC Ct is not within the valid range. • PCC: PASS; all probe check results pass.
ERROR	Presence or absence of MRSA target DNA (*mecA/mecC* or SCC*mec*) cannot be determined. Repeat the test. • *mec* (*mecA/mecC*): NO RESULT • SCC*mec*: NO RESULT • SPC: NO RESULT • PCC: FAIL*; one or more of the probe check results failed. * If the probe check passed, the error was caused by a system component failure.
NO RESULT	Presence or absence of MRSA target DNA (*mecA/mecC* or SCC*mec*) cannot be determined. Repeat the Test. A **NO RESULT** indicates insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred. • *mec* (*mecA/mecC*): NO RESULT • SCC*mec*: NO RESULT • SPC: NO RESULT • PCC: N/A (not applicable). An error caused by the maximum pressure limit exceeding the acceptable range terminates the run prior to probe check.

3. **Reasons to Repeat the Assay**
   a. If any of the test results mentioned above occur, repeat the test according to the instructions in the Retest Procedures section below.
   b. An **INVALID** result indicates that the SPC failed. The sample was not properly processed, or PCR was inhibited.
   c. An **ERROR** result indicates that the Probe Check control failed, and the assay was aborted. Possible causes include: the reaction tube being filled improperly; a reagent probe integrity problem was detected; or the maximum pressure limits were exceeded.
   d. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

4. **Retest Procedure, if required.**
   Repeat the testing procedure using the second collection swab.

5. **RESULT REPORTING:**
   a. Sign onto LIS.
   b. At top of screen select: Result Entry.
   c. Scan the barcode on the worksheet for the batch list.
   d. Select the specimen number you wish to result.
   e. Click the Edit button. Type in the results for the test: positive or negative. Click Verify and Final Verify the result.

6. **A positive *MRSA* result is a Significant Value**
   Positive results on are called on NICU and HH PED9 inpatients.

PROCEDURAL NOTES/LIMITATIONS:

1. Careful compliance with the instructions in this package insert and in Cepheid Sample Collection Device package inserts is necessary to avoid erroneous results.
2. The Xpert MRSA NxG test performance has not been evaluated in patients less than two years of age.
3. The Xpert MRSA NxG test is not intended to diagnose, guide or monitor treatment for MRSA infections, or determine susceptibility to methicillin.
4. As with many diagnostic tests, results from the Xpert MRSA NxG test should be interpreted in conjunction with other laboratory and clinical data available to the clinician and should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions. Results should not be used to guide or monitor treatment for MRSA infections.
5. A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of MRSA.
6. A negative test result does not exclude the possibility of nasal colonization because test results may be affected by improper specimen collection, technical error, sample mix-up, or because the number of organisms in the sample is below the limit of detection of the test.
7. Concomitant cultures are necessary to recover organisms for epidemiology typing or for further susceptibility testing.
8. The Xpert MRSA NxG test provides qualitative results. No correlation can be drawn between the magnitude of the Ct value and the number of cells in an infected sample.
9. Mutations or nucleotide polymorphisms in primer or probe binding regions may affect detection of new or unknown MRSA variants resulting in a false negative result.
10. An Xpert MRSA NxG test positive result does not necessarily indicate intervention eradication failure since nonviable DNA may persist. A negative result following a previously positive test result may or may not indicate eradication success.
11. Because the detection of MRSA is dependent on the quantity DNA present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.

12. The Xpert MRSA NxG test may generate a false positive MRSA (**MRSA DETECTED**) result when testing a nasal specimen with a mixture of organisms containing both methicillin-resistant coagulase-negative *Staphylococcus* and an empty cassette SA.
13. The Xpert MRSA NxG test may generate a false negative result (**MRSA NOT DETECTED**) in the event of a co-colonization that contains both methicillin-resistant *Staphylococcus aureus* (MRSA) and an empty cassette *Staphylococcus aureus* (SA). This may occur in rare cases when the titer of an empty cassette SA organism is substantially higher than that of the MRSA organism.
14. Assay interference may be observed in the presence of Nasonex (≥50% v/v), Flonase (≥50% v/v), and Beconase (≥40% v/v).

REFERENCES:

1. Package insert, Xpert® *MRSA NxG, current version*. Cepheid AB Sunnyvale, CA
2. *GeneXpert Dx System Operator Manual,* Cepheid AB Sunnyvale, CA