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|  | **LABORATORY DEPARTMENT****POLICY AND PROCEDURES** | **Department: Serology** |
| **Number:** **1161.6.Sero.gu.reg12/2014** |

**METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) NASAL COMPLETE**

**BY CEPHEID REAL-TIME™ PCR**

PURPOSE

 Xpert MRSA Assayis PCR based method that analyzes for the *mecA* gene (SCCmec) in the detection of Methicillin Resistant *Staphlylococcus aureus (MRSA)*

INTENDED USE

The Cepheid Xpert MRSA Assay performed in the GeneXpert® Dx System (Xpert MRSA) is a qualitative *in vitro* diagnostic test designed for rapid detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs in patients at risk for nasal colonization. The test utilizes automated real-time polymerase chain reaction (PCR) to detect MRSA DNA. The Xpert MRSA Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. The Xpert MRSA Assay is not intended to diagnose MRSA nor to guide or monitor treatment for MRSA infections. Concomitant cultures are necessary only to recover organisms for epidemiological typing or for further susceptibility testing.

LIMITATIONS

Results from the Xpert MRSA 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, 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 is necessary to avoid erroneous results.

Because the detection of MRSA is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.

Rerunning the Xpert MRSA when results are INVALID, ERROR, and NO RESULT should depend on practices and policies within each facility. Alternate procedures (i.e. culture using selective agar plates with or without overnight incubation in a selective enrichment broth) should be available. For culturing remaining swab specimens should be placed in appropriate transport systems and cultured within 4 days.

A positive test result does not necessarily indicate the presence of viable organism. It is, however, presumptive for the present of MRSA. Testing with Xpert MRSA Assay should be used as an adjunct to other methods available. 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. Mutations of polymorphisms in primer or probe binding regions may affect detection of new or unknown MRSA variants resulting in a false negative result.

REAGENTS AND INSTRUMENTS

Material Provided

The Xpert MRSA (GXSACOMP-10) contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert MRSA Assay Cartridges with integrated reaction tubes 10

Bead 1 (freeze-dried) 1 per cartridge

Contains:

Polymerase

dNTPs

BSA (bovine serum albumin)

Bead 2 (freeze dried) 1 per cartridge

Contains:

Primers

Probes

BSA (bovine serum albumin)

Bead 3 (freeze-dried) 1 per cartridge

Contains:

Sample Processing Control (SPC) ~6000 noninfectious sample preparation control spores

Xpert MRSA reagent pouches 10 per box

Contains:

Elution Reagent (Guanidinium Thiocyanate and surfactants) pouch 1 x 1.5 ml per

Reagent 1 (Sodium hydroxide) pouch 1 x 3.0 ml per

Reagent 2 (Tris Buffer, EDTA and surfactants) per pouch 1 x 2.75 ml

NOTES

Material Safety Data Sheets (MSDS) for all reagents provided in this assay are available upon request from Cepheid Technical Support.

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 not co-mingling of the material with other animal materials.

1. STORAGE AND HANDLING
2. Store the Xpert MRSA cartridges and reagents at 2-28°C.
3. Do not use reagents or cartridges that have passed the expiration date.
4. Do not open a cartridge until you are ready to perform testing.
5. Use the cartridge and reagents within 30 minutes after opening the package.
6. Do not use any reagents that have become cloudy or discolored.

MATERIALS REQUIRED BUT NOT PROVIDED

GeneXpert® Dx System (catalog number varies by configuration): GeneXpert® instrument, computer, barcode wand reader and Operator Manual

sterile transfer pipettes

Sterile Printer (See *GeneXpert® Dx System Operator Manual* for compatibility

Cepheid Sample Collection Device (part number 900-0370)

Vortex mixer

Disposable gauze

MATERIALS AVAILABLE BUT NOT PROVIDED

KWIK-STIK™ from MicroBioLogics catalog # 0158 MRSA as positive control (ATCC® # 700699) , Positive MSSE control: 0360MSSAand # 0371 MSSE (Methicillin sensitive *Staphylococcus epidermis*) as negative control (ATCC® #12228).

SPECIMEN COLLECTION AND TRANSPORT

To obtain adequate specimen follow the instructions in this section closely.

Open the Cepheid Collection Device by peeling back the outer packaging.

Ask the patient to tilt his/her head back. Insert dry swabs approximately 1-2 cm into each nostril.

Rotate the swabs against the inside of the nostril for 3 seconds. Apply slight pressure with a finger on the outside of the nose to help assure good contact between the swab and the inside of the nose.

Using the same swabs repeat for the second nostril, trying not to touch anything but the inside of the nose.

Remove the plastic transport tube. Twist off the tube cap and discard it. Place the swabs into the plastic transport tube. The swabs should go all the way into the tube until they rest on top of the sponge at the bottom of the tube. Make sure the red cap is on tightly. **Note: The swabs should stay attached to the red cap at all times**.

Label the plastic transport tube with patient ID and send to the laboratory.

Store swab specimen at room temperature (15-30°C) if it will be processed within 24 hours, otherwise store swab at 2-8°C. The swab specimen is stable up to 5 days when stored at 2-8°C.

Preparing the Cartridge

**Important:** Start the test within 15 minutes of adding the reagents to the cartridge.

**Note:** Use only one of the swabs. The second swab is required for repeat testing.

To Add the Sample and Reagent into the Cartridge (Xpert MRSA)

1. Remove the cartridge and reagents from the package.
2. Remove the swabs from the transport container and then remove one swab from the red cap.
3. Insert the swab into the tube containing the Elution Reagent (black cap).

**Note:** use sterile gauze to minimize risks of contamination.

1. Hold the swab by the stem near the rim of the tube, lift the swab a few millimeters from the bottom of the tube and push the tem against the edge of the tube to break it. Make sure the swab is short enough to allow the cap to close tightly.
2. Close the lid and vortex at high speed for 10 seconds.
3. Open the cartridge lid. Using a sterile transfer pipette transfer the entire contents of the Elution Reagent (black cap) to the “S” chamber of the GeneXpert cartridge.
4. Close the cartridge lid.

Starting the Test

**Important: Before you start the test make sure the Xpert MRSA Assay definition is imported into the software.**

This section lists the basic steps of running the test. For detailed instructions see the *GeneXpert® Dx System Operator Manual*.

Turn on the computer and then turn on the GeneXpert® Dx instrument.

1. On the Windows® desktop double click the GeneXpert® shortcut icon.
2. Logon to the GeneXpert® Dx System software using your user name and password.
3. In the GeneXpert® Dx System window click **Create Test**. The Scan Cartridge Barcode dialog box appears.
4. Scan the barcode on the Xpert MRSA 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.
5. In the **Sample ID** box scan or type the sample ID. 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.
6. Click **Start Test**. In the dialog box that appears type your password.
7. Open the instrument module door with the blinking green light and load the cartridge.
8. Close the door. The test starts and the green light stops blinking. When the test is finished the light turns off.
9. Wait until the system releases the door lock before opening the module door and removing the cartridge.
10. The used cartridges should be disposed in the appropriate specimen waste containers according to your institution’s standard practices.

QUALITY ASSURANCE AND QUALITY CONTROL

Each test includes a Sample Processing Control (SPC) and probe check (PCC).

Positive and negative controls should be tested once for each new lot/shipment or every 30 days, whichever is more frequent.

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 MRSA. The SPC verifies that lysis of MRSA 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 – KWIKSTIK™ (MicroBioLogics catalog # 0158 MRSA as positive control and # 0371 MSSE as negative control) may be used for training, proficiency testing and external QC of the GeneXpert Dx System. External controls may be used in accordance with local, state, federal accrediting organizations, as applicable. Follow the MicroBioLogics external control procedure described below:

Tear open the pouch at notch and remove the KWIK-STIK.

Pinch the bottom of the ampoule in the cap to release the hydrating fluid.

Hold vertically and tap to facilitate flow of fluid through shaft into bottom of unit containing pellets.

To facilitate dissolution of the lyophilized cell pellet crush the pellet and gently pinch the bottom chamber.

Pull apart the KWIK-STIK to release the swab and insert the swab into the tube containing the Elution Reagent (black cap).

The KWIK-STIK swab is now ready for Xpert MRSA testing.

INTERPRETATION OF 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:

**MRSA POSITIVE**

MRSA target DNA is detected (presumptive positive for MRSA colonization)

* MRSA POSITIVE – The MRSA target has a Ct within the valid range and endpoint above the minimum setting.
* SPC – NA (not applicable); SPC is ignored since MRSA amplification may compete with this control.
* Probe Check – PASS; all probe check results pass.

**MRSA NEGATIVE**

MRSA target DNA is not detected (presumed not colonized with MRSA), SPC meets acceptance criteria.

* MRSA NEGATIVE – MRSA target DNA is not detected.
* SPC – PASS; SPC has a CT within the valid range and endpoint above the endpoint minimum setting.
* Probe Check – PASS; all probe check results pass.

**INVALID**

Presence or absence of MRSA cannot be determined, repeat test with extra swab. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

* MRSA INVALID – Presence or absence of MRSA DNA cannot be determined.
* SPC – FAIL; MRSA target result is negative and the SPC Ct is not within valid range and endpoint below minimum setting.
* Probe Check – PASS; all probe check results pass.

**ERROR**

Presence or absence of MRSA cannot be determined, repeat the test with extra swab. The Probe Check control failed probably due to reaction tube was filled improperly, a probe integrity problem was detected or because the maximum pressure limits were exceeded.

**NO RESULT**

Presence or absence of MRSA cannot be determined, repeat test with extra swab. Insufficient data were collected to produce a test result (for example: the operator stopped a test that was in progress).

* MRSA – NO RESULT
* SPC – NO RESULT
* Probe Check – NA (not applicable)

INSTRUMENT MAINTENANCE

Although the system is designed to prevent cross-contamination and ensure accurate results the instrument must be checked and cleaned periodically as a precautionary measure. The following table lists the manufacturer’s recommended maintenance tasks and how often they should be performed.

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| **TASK** | **FREQUENCY** | **REFERENCEd SECTION IN USERS MANUAL** |
| Disinfect the Instrument Surfaces | Monthly | Section 10.2 |
| Disinfect the Cartridge Bay Interior | Monthly | Section 10.3 |
| Disinfect he Syringe Plunger Rod | Monthly | Section 10.4 |
| Calibrate the Instrument | Annually, at 2000 tests per module, or as indicated by the Xpert Check Software | Section 10.5 |
| Check Module Reported Calibration | When requested by Cepheid Support | Section 10.6 |
| Perform a Self-Test Manually | As necessary | Section 10.7 |

NOTES

1. Disinfect the instrument surfaces using paper towels soaked in a freshly prepared 10% bleach solution, wait 10 minutes and wipe off the remaining bleach solution with 70% alcohol. Dispose of paper towels in appropriate lab waste.
2. Disinfect the cartridge bay using Dacron or cotton swabs soaked in a freshly prepared 10% bleach solution, wait 10 minutes and wipe off the remaining bleach solution with 70% alcohol. Dispose of paper towels in appropriate lab waste.
	1. **\*\*\*Important Note\*\*\*: Do NOT touch the slit on the I-CORE module into which the cartridge reaction tube is inserted. Getting liquid inside of the I-CORE module can damage the module.**
3. Disinfect the plunger rod using Dacron or cotton swabs soaked in a freshly prepared 10% bleach solution by using the following procedure:
	1. In the GeneXpert Dx System window click “Maintenance” on the toolbar
	2. The maintenance window appears
	3. On the “Maintenance” menu click “Plunger Maintenance” and the plunger maintenance dialog box appears
	4. In the module tab of the Plunger Maintenance dialog box click “Clean”. The “Clean” button will change to “Move Up”. In the instrument the plunger rod in the selected module lowers into the cartridge bay.
	5. Clean the plunger rod with a freshly prepared 10% bleach solution, wait 5 minutes and wipe off the remaining bleach solution with swabs dipped in 70% alcohol (repeat the 70% alcohol step twice).
	6. In the plunger maintenance dialog box click “Move Up” and the plunger rod will move back to the resting position.
	7. **Fresh swabs must be used for each individual bay.**
4. Generally, instrument calibration is recommended annually or once every 2000 tests. However, Cepheid software may prompt an instrument calibration prior to this. To check whether the instrument requires calibration:
	1. In the maintenance window look at the “I-CORE Starts Since Cal” column and module recorder calibrate date.
	2. On the maintenance menu click “Module Reports”.
	3. Check the date and number of tests.
	4. If needed contact Cepheid Technical Support to schedule a calibration.

ATTACHMENTS MRSA Quick Reference

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**METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) DETECTION**

**BY CEPHEID REAL-TIME™ PCR**

**(MRSA QUICK REFERENCE)**

PROCEDURE

Remove only one swab from the transport container. Save the second swab for repeat testing.

Insert the swab into the tube containing the Elution Reagent.

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

Open the cartridge lid. Using a sterile transfer pipette transfer the entire contents of the Elution Reagent to the “S” chamber of the GeneXpert cartridge, **avoiding bubbles.**

Close cartridge lid.

**Start the test within 15 minutes of adding the reagents to the cartridge**.

On the Windows® desktop double click the GeneXpert® shortcut icon and log on to the GeneXpert® Dx System software.

In the GeneXpert® Dx System window click “*Create Test”*. Scan the barcode on the sample (or click on the manual entry box to enter the sample name.)

Scan the barcode on the Xpert MRSA cartridge once which enters the Assay, Reagent Lot Id, Cartridge SN, and Expiration Date.

Click “*Start Test”.* In the dialog box that appears type your password.

Open the instrument module door with the blinking green light and load the cartridge.

**Close the door and hold it closed until you hear the door lock. Failing to do so will result in a system error.** The test starts and the green light stops blinking. When the test is finished the light turns off.

The results are interpreted by the GeneXpert® Dx System and will be shown in the **“***View Results***”** window.

Enter the results in the LIS.

**REASONS TO REPEAT THE ASSAY**

“Invalid “ result indicates that the controls SPC failed

“Error” result indicates that the Probe Check control failed

“Invalid” result indicates that insufficient data were collected , (for example: the operator stopped a test that was in progress).