TITLE: XPERT SA NASAL COMPLETE

PRINCIPLE / PURPOSE: The Cepheid Xpert SA Nasal Complete Assay performed on the GeneXpert System is a qualitative in vitro diagnostic test designed for rapid detection of *Staphylococcus aureus* (SA) and 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/SA DNA. The Xpert SA Nasal Complete Assay is intended to aid in the prevention and control of MRSA/SA infections in healthcare settings. The Xpert SA Nasal Complete Assay is not intended to diagnose, guide or monitor treatment for MRSA/SA infections, or provide results of susceptibility to

Methicillin. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

SCOPE: To provide instruction on the processing and reporting of specimens submitted for SA Nasal Complete testing.

SPECIMEN:

Type: Nasal swab transported in the Copan Transystem Culture Swab Transport System.

Handling Conditions:

Follow safety procedures for working with chemicals and handling biological samples.

EQUIPMENT AND MATERIALS:

Equipment:

Gene Xpert Cepheid analyzer

Vortex

Safety Shield/Eye protection

Gloves

Lab Coat

Materials:

Xpert SA Nasal Complete Cartridges

Xpert SA reagents

Disposable, sterile transfer pipettes

Gauze

Storage Requirements:

1. Store cartridges and reagents at 2-28°C.
2. Do not use reagents or cartridges that have passed the expiration date.
3. Specimens are stable at room temperature for 24 hours. If testing is not performed within 24 hours, specimens are stable for 5 days refrigerated.
4. The cartridge and reagents are stable for up to 7 days after opening the package.

CALIBRATION:

Calibration of the individual modules is required annually and is performed by the Cepheid service rep.

QUALITY CONTROL:

External QC

External controls are to be performed upon receipt of every new shipment and upon the

implementation of every new lot number. Record lot number, expiration date, and control results on the Xpert QC log.

Positive MRSA control = *S aureus* ATCC 700699

Positive MSSA control = S. aureus ATCC 25923

Negative MRSA/MSSA control = *S. epidermidis* ATCC 12228

QC Procedure:

1. After incubation and if there is adequate growth remove two of the red swabs and label one with positive control and the other as negative control.
2. Open the positive control swab and then grasp the red cap.
3. Open the plate of Staph aureus ATCC 700699 for MRSA positive control and obtain some of the organism on both swabs.
4. Repeat this with Staph aureus ATCC 25923 for MSSA positive control and with the negative control swab using Staph aureus ATCC 12228.
5. Using the bottle of ethanol, spray the area behind the plastic shield and using a 4 x 4 gauze clean the area to prevent contamination.
6. Take off your gloves and wash and dry your hands.
7. Don a new pair of gloves
8. Remove the cartridge and elution reagent from the package.
9. Remove one swab from the red cap of your positive control and return the second swab to the transport container.
10. Insert the swab into the tube containing the elution reagent
11. Using a sterile gauze hold the swab by the stem near the rim of the tube and lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the tube to break it. Make sure the swab is short enough to allow the cap to close tightly.
12. Close the lid and vortex at high speed for 10 seconds.
13. 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. Allow sample to trickle down the side of the “S” chamber by holding the pipette against the side of the chamber. This minimizes the amount of bubbles.



1. Close the cartridge lid.
2. Label the side of the cartridge with MRSA 700699.
3. Repeat the steps above for the MSSA 25923 and MRSA/MSSA 12228 negative control.
4. In the GeneXpert window, click Create Test. A window pops up that says “Please scan sample ID barcode”. Using the mouse, left click on the “Manual Entry” box.
5. In the Sample ID box, type the control ID "MRSA 700699”. Make sure you type the correct control ID.
6. Scan the barcode on the cartridge.
7. Click Start Test. In the dialog box that appears, type your password if needed.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking.
10. Using the bottle of ethanol, spray the area behind the plastic shield and using a 4 x 4 gauze clean the area to prevent contamination.
11. Take off your gloves and wash and dry your hands.
12. Don a new pair of gloves
13. In the GeneXpert window, click Create Test. A window pops up that says “Please scan sample ID barcode”. So using the mouse, left click on the “Manual Entry” box.
14. Repeat steps 17 – 26 with the other 2 controls (25923 and 12228).

PROCEDURE: (PATIENT)

1. Preparation
2. Using the bottle of ethanol, spray the area behind the plastic shield and using a

4 x 4 gauze clean the area to prevent contamination.

1. Take off your gloves and wash and dry your hands.
2. Don a new pair of gloves
3. Remove the cartridge and elution reagent from the package.
4. Two swabs are located in each culturette. Only use one of the swabs. The second swab is required for repeat testing.
5. Remove one swab from the red cap and return the second swab to the transport container.
6. Insert the swab into the tube containing the elution reagent
7. Using a sterile gauze hold the swab by the stem near the rim of the tube and lift the swab a few millimeters from the bottom of the tube and push the stem against the edge of the tube to break it. Make sure the swab is short enough to allow the cap to close tightly.
8. Close the lid and vortex at high speed for 10 seconds.
9. 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. Allow sample to trickle down the side of the “S” chamber by holding the pipette against the side of the chamber. This minimizes the amount of bubbles.



1. Close the cartridge lid.
2. Starting the Test
3. Turn on the computer, and then turn on the GeneXpert instrument if it is not already on.
4. On the Windows® desktop, double-click the GeneXpert shortcut icon. If the computer is already on this step will not need to be performed.
5. Log on to the GeneXpert System software using your user name and password.
6. In the GeneXpert window, click Create Test. A window pops up that says “Please scan sample ID barcode”. Using the barcode reader scan the patient’s barcode accession number. If you need to manually enter the patient’s accession number or if the barcode accession number is not reading, use the mouse and left click on the “Manual Entry” box. Type the accession number and click on “OK”
7. 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.
8. Type the patient’s name in the comment box.
9. A box pops up labeled “Please scan cartridge barcode”. Using the barcode reader, scan the barcode on the Xpert MRSA Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and expiration Date.
10. Click Start Test. In the dialog box that appears, type your password if needed.
11. Open the instrument module door with the blinking green light and load the patient’s cartridge.
12. Close the door. The test starts and the green light stops blinking.
13. Proceed to the next patient making sure to clean the work area behind the plastic shield with ethanol and changing gloves. Your hands must be washed between glove changes.
14. Once testing is complete, fill in the patient log and record in LIS.
15. The used cartridges should be disposed in the appropriate specimen waste containers according to ARMS’s standard practices.

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

* Sample Processing Control (SPC) – ensures the sample was correctly processed. The SPC contains of spores of Bacillus globigii in the form of a dry spore cake that is included in each cartridge to verify adequate processing of SA Complete. Assay sample. The SPC verified that lysis of SA 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 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.

INTERPRETATION & REPORTING RESULTS:

Decodes:

MRN – Negative - MRSA target DNA NOT DETECTED.

MRP – Positive - MRSA target DNA DETECTED.

SRN – Negative - MSSA target DNA NOT DETECTED.

SRP – Positive – MSSA target DNA DETECTED.

INV – UNABLE TO INTERPRET RESULTS.

VALID – CONTROL VALID

MRSA POSITIVE; SA POSITIVE

MRSA target DNA detected; SA target DNA detected.

• All MRSA targets (*spa*, *mecA* and SCC*mec*) have a Ct within the valid range and endpoint above the threshold setting.

• SPC – NA (not applicable); SPC is ignored since MRSA amplification may compete with this control.

• Probe Check – PASS; all probe check results pass.

A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of

MRSA or SA.

MRSA NEGATIVE; SA POSITIVE

MRSA target DNA not detected; SA target DNA detected.

• SA target (*spa*) has a Ct within the valid range and endpoint above the threshold setting. Target DNA for SCC*mec* is not detected

and target DNA for *mecA* is or is not detected.

• SA target (*spa*) has a Ct within the valid range and endpoint above the threshold setting. Target DNA for *mecA* is not detected and

target DNA for SCC*mec* is detected (empty cassette variant).

• SPC – NA (not applicable); SPC is ignored since SA amplification may compete with this control.

• Probe Check – PASS; all probe check results pass.

An MRSA NEGATIVE; SA POSITIVE test result does not preclude MRSA nasal colonization.

MRSA NEGATIVE; SA NEGATIVE

SA target DNA not detected.

• SA target (*spa*) DNA is not detected. Target DNA for *mecA* may or may not be detected; target DNA for SCC*mec* may or may not

be detected.

• SPC – PASS; SPC has a Ct within the valid range and endpoint above the threshold setting.

• Probe Check – PASS; all probe check results pass.

An MRSA NEGATIVE; SA NEGATIVE test result does not preclude MRSA or SA nasal colonization.

A false negative for MRSA (a result of “MRSA NEGATIVE; SA POSITIVE” instead of “MRSA POSITIVE; SA POSITIVE”)

could be obtained if both MRSA and SA are present in the sample at an MRSA:SA ratio of 1:1103 or greater.

INVALID

Presence or absence of MRSA and SA target DNA cannot be determined. Repeat test according to instructions in the section below.

• SPC – FAIL; SPC target result is negative, and the SPC Ct is not within the valid range and endpoint is below the threshold

setting.

• Probe Check – PASS; all probe check results pass.

ERROR

Presence or absence of MRSA and SA target DNA cannot be determined. Repeat test according to instructions in the section below.

• MRSA and SA targets – NO RESULT

• SPC – NO RESULT.

• Probe Check – FAIL\*; one or more of the probe check results failed.

\*If the probe check passed, the error was likely caused by the maximum pressure exceeding the acceptable range.

Procedures for Abnormal Results:

Repeat the test using a new cartridge (do not re-use the cartridge) and the second swab if any of the following:

1. INVALID - which indicates the controls SPC failed. The sample was not properly processed or PCR is inhibited
2. ERROR – which indicates that the probe check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.
3. NO RESULT – indicates that insufficient data was collected. For example, the operator stopped a test that was in progress.

If the second swab yields the same results then call the doctor or nurse and document the physician’s name or the first and last name of the nurse with the date and time.

All of these results must be verified by repeat testing and called to the appropriate personnel which will be documented. Then the test must then be cancelled

PROCEDURE NOTES:

1. Treat all biological specimens, included used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with universal precautions.
2. Follow safety procedures for working with chemicals and handling biological samples.
3. Do not substitute reagents with other reagents.
4. Do not open the cartridge lid except when adding sample and reagents.
5. Do not use a cartridge that has been dropped or shaken after you have added the sample and reagents.
6. Do not use a cartridge that has a damaged reaction tube.
7. Each single-use cartridge is used to process one test. Do not reuse spent cartridges.
8. Dispose of used cartridges according to your institution safety guidelines for hazardous material.
9. Store kits at 2 - 28°C
10. Do not open a cartridge package until you are ready to perform the test.

Interfering Substances:

Inhibitory effects on the SA Nasal Complete Assay resulting in invalid test results were observed in the presence of inhaled nasal steroids Flonase and Nasonex.

Inhibitory effects on the SA Nasal Complete Assay resulting in false negative test results were observed in the presence of inhaled nasal steroids Flonase

and Nasonex.

RELATED PROCEDURES:

Specimen Collection and Processing.

Specimen Processing and Labeling.

QM-195 Proficiency Testing.

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SOP Number: MICRO-726

SOP Title: SA Nasal Complete

Written By: Jacee Farmer

Manual in which Hard Copy of this SOP is located: Microbiology Manual III

Distribution: none

Supersedes Procedure: none

SOP CHANGE CONTROL

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