Assay Training: Xpert® MRSA NxG

Technical Training for US-IVD product only
Training Agenda

- Xpert MRSA NxG Training
  - Reagents
  - Sample Collection
  - Kit storage and handling
  - Preparing cartridge
- Quality Control
- Results Analysis
- Discussion and Q&A
Xpert MRSA NxG Training Objectives

At the end of the training, the user will be able to:

- Store and handle the Xpert MRSA NxG cartridge kits and sample collection kits
- Follow proper laboratory safety precautions
- Identify appropriate specimen types and transport specimens
- Prepare a cartridge and run the assay
- Report and understand the various software-generated results
- Understand the assay control strategy
MRSA
What is MRSA?

**Resistance and decolonization**

- Methicillin-resistant *S. aureus* (MRSA) are strains of *S. aureus* that acquired resistance to semi-synthetic penicillins, such as methicillin, by acquisition of a *mec* gene\(^1\)
- Nasal decolonization with mupirocin, 2% ointment three times-a-day for five days\(^2\) and daily chlorhexidine body washes for five days

**S. aureus treatment:**
- Oxacillin, first-generation cephalosporin or for wounds, fluoroquinolone, or Trimethoprim-sulfa

**MRSA treatment:**
- Vancomycin, daptomycin, or linezolid

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\(^1\) Investigation of Specimens for Screening for MRSA, NHS 2014

The Need:

**MRSA is an evolving threat that requires uncompromising solutions**

- Every year, new strains emerge and threaten to erode recent hard-fought gains in HAI prevention.
- Active surveillance is proven to help reduce HAI rates, but sensitivity and speed are essential.
- Effective infection control programs require an on-demand test that meets clinician and laboratory needs.

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The Solution:

The #1 on-demand test for MRSA colonization is now improved

- Expanded coverage demonstrated using an extensive library of over 196 MRSA strains from around the world\(^5\)
- Updated primers detect both \textit{mecA} and \textit{mecC} strains and reduce false-positive results due to “empty cassettes”\(^5\)
- Validated for use with both rayon swabs and ESwab\(^\text{TM}\) (Copan)\(^5\)

\(^5\) Xpert MRSA NxG Package Insert.
Xpert® MRSA
The Cepheid Solution

- Detection of *mecA*, *mecC*, and *SCCmec* genes
- Two controls for each individual sample
  - Sample Processing Control (SPC)
  - Probe Check Control (PCC)
- High sensitivity and specificity
- Simple and easy to use
  - Closed cartridge system
- EAT (Early Assay Termination)
- On-demand results 24/7
- Random access
The Xpert® MRSA NxG Assay, performed on the GeneXpert® Instrument Systems, is a qualitative in vitro diagnostic test intended for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA directly from nasal swabs in patients at risk for nasal colonization.

The test utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA-specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA.

The Xpert MRSA NxG Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. The Xpert MRSA NxG Assay is not intended to diagnose, guide, or monitor treatment for MRSA infections, or provide results 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.
System and Reagent Requirements

GeneXpert Systems
- GeneXpert Software Version 4.3 or higher

Test Kits (US-IVD)
- GXMRSA-NXG-10
- GXMRSA-NXG-120

Materials Required but not Provided
- One of the following Collection Devices:
  - ESwab Collection and Transport System
    (Copan #480CE, Copan #480C, or BD ESwab collection kit #220245)
    - When using the ESwab Collection kit, 300ul disposable, sterile pipette
  - Copan Dual Swab and Transport Systems
    (Copan #139C LQ STUART or Cepheid #900-0370)
    - Sterile gauze
- Disposable, sterile transfer pipettes
- Vortex mixer
# Xpert MRSA NxG Kit Contents

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<th>Table Title</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Xpert MRSA NxG Assay</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Catalog Number | GXMRSA-NXG-10  
GXMRSA-NXG-120 |
| Tests Per Kit | 10 or 120 |
| Cartridge Contents | Reagent beads  
Liquid Reagents |
| Kit CD | Assay Definition File (ADF)  
Assay Import Instructions  
Package Insert (PDF) |
| Reagent Vials per kit | 10 or 120 |
| Storage | 2-28 °C |
Good Laboratory Practice

PCR laboratory setup
- Cartridge/reagent preparation → Sample addition → Detection

Specimen and reagent storage
- Store specimens separately from reagents to prevent reagent contamination.

Equipment
- Use filtered pipette tips, when needed, for QC dilutions.
- Follow the manufacturer’s recommendation for calibration and maintenance of the lab equipment.
Good Laboratory Practice, cont’d

**Housekeeping**
- Clean work surfaces with a final concentration of 1:10 dilution of household bleach* and then 70% ethanol or 70% denatured ethanol. Wipe work surfaces dry.

**Personnel**
- Wear clean lab coats and gloves.
- Change gloves between processing samples.

**Lab bench area**
- Clean the lab bench area routinely.
- Keep the back of the instrument dust free.

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*Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*
Xpert MRSA NxG Kit Storage and Handling

- Store test kits at 2-28°C. Do not use expired cartridges.

- Each single-use cartridge is used to process one test. Do not reuse processed cartridges.

- Do not open a cartridge until ready to use.
  - Start the test within 30 minutes of adding the sample to the cartridge.

- Avoid cross contamination during sample handling steps.
  - Change gloves if they come in contact with specimen or appear to be wet.
  - Change gloves before leaving work area and upon entry into work area.

- Do not use a cartridge that has been dropped or shaken after the sample has been transferred to the cartridge. Shaking or dropping the cartridge after opening the lid may yield invalid results.

- Do not use a cartridge that has a damaged reaction tube.

- Do not use a cartridge that has leaked.
Warning and Precautions

• Do not shake the cartridge
• Do not use a cartridge...:
  – if it appears wet, has leaked or if the lid seal appears to have been broken
  – if it appears damaged
  – that has been dropped after removing it from packaging
  – that has been dropped or shaken after adding the sample to it
  – that has a damaged reaction tube
  – that has been used: each cartridge is single-use to process one test
• Do not reuse disposable pipettes
• Do not reuse disposable swabs
• Dispose Xpert MRSA NxG Assay cartridges and reagents according to your institution’s and country’s guidelines for disposal of hazardous materials
Specimen Collection
Specimen Collection Devices to be used with MRSA NxG Test

Dual Swab and Transport Systems
(Cepheid #900-0370 or Copan #139C LQ STUART)

ESwab Collection and Transport System
(Copan #480C, Copan #480CE or BD #220245)
Specimen Collection and Storage (dual swab)

Nasal Specimen Collection Protocol for use with Xpert® assays:

1. Swabs to be used: Cepheid Sample Collection Device (Part No. 900-0270) or Copan Dual Swab and Transport Systems (13OC LQ STUART).
   - Note: The double-swab is not packaged in the transport tube.
2. Insert the dry swabs 1-1.5 cm into the nostril.
   - Note: The swabs must stay attached to the red cap throughout the procedure.
3. Rotate swabs against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril.
   - Do not insert the swabs more than 1-1.5 cm.
4. Repeat Step 3 on the other nostril with the same swabs, using external pressure on the outside of the other nostril.
   - To avoid specimen contamination, do not touch the swab tips to anything other than the inside of the nostril.
5. Remove and discard the cap on the transport tube and place the swabs into the tube pushing the red cap down completely.
6. Specimens that are tested within 24 hours can be kept at room temperature. For longer storage, refrigerate the specimen at 2-8°C. Specimens stored at 2-8°C are stable for up to 7 days.
Specimen Collection and Storage (ESwab)

Nasal Specimen Collection Protocol for use with Xpert® assays:

- **Xpert MRSA NxG**

1. Swabs to be used: Liquid Amies Elution Swab (ESwab) Collection and Transport System (Copan 480C, Copan 480CE or BD 220248).
   
   Note: The swab is not packaged in the transport tube.

2. Insert the dry swab 1-1.5 cm into the nostril.

3. Rotate swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril.
   
   Do not insert the swab more than 1-1.5 cm.

4. Repeat Step 3 on the other nostril with the same swab, using external pressure on the outside of the other nostril.
   
   To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

5. Remove the cap on the transport tube and place the swab into the tube and break it against the edge of the tube, replace the cap making sure it is secure.

6. Specimens that are tested within 24 hours can be kept at room temperature. For longer storage, refrigerate the specimen at 2-8°C. Specimens stored at 2-8°C are stable for up to 7 days.
Xpert MRSA NxG Cartridge Preparation - dual swab

**Xpert® MRSA NxG Cartridge Preparation Using dual swab**

1. Obtain one Xpert cartridge and one Elution Reagent vial for each sample.
2. Insert a single swab into the Elution Reagent vial.
3. Break the swab at the score mark near the opening of the vial.
4. Recap the Elution Reagent vial and vortex at high speed for 10 seconds.
5. Open the Xpert cartridge lid.
6. Using a transfer pipette (not supplied), transfer the entire contents of the vial to the Sample Chamber of the cartridge.
7. Empty the pipette into the sample chamber.
8. Close the Xpert cartridge lid.
9. Start the test within the timeframe specified in the package insert.

Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.

US-IVD. For in-vitro diagnostic use
Xpert MRSA NxG Cartridge Preparation - ESwab

**Xpert® MRSA NxG Cartridge Preparation**

Using an ESwab

**ESwab Collection and Transport System in Liquid Amies media**
(Copan 480C, Copan 480CE or BD ESwab Collection Kit Part # 220245)

**Refer to the package insert for detailed instructions, precautions, and warnings.**

Cepheid Technical Support
US office
(866) 638-3222, Option 2
techsupport@cepheid.com
European office
+33 963 82 53 19
support@cepheid europe.com

1. Obtain one Xpert cartridge and one Elution Reagent vial for each sample.
2. Vortex the swab sample at high speed for 5 seconds.
3. Using a transfer pipette (not supplied), transfer 300 µL of the liquid sample into the Elution Reagent vial.
4. Recap the Elution Reagent vial and vortex at high speed for 10 seconds.
5. Open the Xpert cartridge lid.
6. Using a transfer pipette (not supplied), transfer the entire contents of the vial to the Sample Chamber of the cartridge.
7. Close the Xpert cartridge lid.
8. Start the test within the timeframe specified in the package insert.

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US-IVD. For in-vitro diagnostic use
Automated Xpert MRSA NxG Load Test Steps

1. Add the sample to the cartridge.
2. Place the cartridge into the instrument.
3. Nucleic acids are purified.
4. Purified nucleic acids mix with PCR reagents.
5. Simultaneous amplification and detection occurs.
6. Results are ready to view.
Waste Disposal

- Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents. Check state and local regulations as they may differ from federal disposal regulations.
- This material may exhibit characteristics of hazardous waste requiring specific disposal requirements.
- Institutions should check their country hazardous waste disposal requirements.
Quality Control

Refer to the Package Insert for complete details
Cepheid Assay Control Strategy

- Each Xpert cartridge is a self-contained test device.
  - Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes within each cartridge.
    - Probe Check Control: PCC
    - Sample Processing Control: SPC
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. The PCC passes if it meets the assigned acceptance criteria.
- The readings are compared to default settings established by Cepheid.
- The Probe Check controls for:
  - Missing Target Specific Reagent (TSR) and/or Enzyme Reagent beads (EZR), which contain all primers, probes, and internal control template
  - Incomplete reagent reconstitution
  - Incomplete reaction tube fill
  - Probe degradation
- If the Probe Check fails, an ERROR test result will be reported.
Sample Processing Controls - SPC

• The Sample Processing Control (SPC) assesses the effectiveness of the sample preparation steps, including reaction tube filling.

• The SPC controls for:
  – Missing primer/probe or enzyme beads
  – Incomplete reagent reconstitution
  – Incomplete reaction tube fill
  – Enzyme degradation
  – Sample lysis, nucleic acid extraction, and integrity of nucleic acid
  – Sample inhibition

• The SPC can be negative or positive in an analyte-positive sample.

• If the SPC fails in an analyte-negative sample, an INVALID test result will be reported.
# Commercially Available External Controls

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Description</th>
<th>Configuration</th>
<th>Storage</th>
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</thead>
<tbody>
<tr>
<td>NATMRSA-6MC</td>
<td>MRSA Positive</td>
<td>0.5 mL x 6 vials</td>
<td>2-8°C</td>
</tr>
<tr>
<td>NATMSSE-6MC</td>
<td>MRSA/SA Negative</td>
<td>0.5 mL x 6 vials</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>

www.ZeptoMetrix.com

1. Vortex the NATtrol control for 5-10 seconds.
2. Pipette 100 µL of NATtrol control into 2 mL of Elution Reagent.
3. Vortex the Elution Reagent vial for 5-10 seconds.
4. Use a transfer pipette (not provided) to transfer the entire contents from the Elution Reagent vial into the Sample Chamber of the cartridge.
5. Close the cartridge lid and start the test
Results Analysis

Refer to the Package Insert for complete details
How to detect MRSA using molecular diagnostic methods

- Resistance to methicillin and other ß-lactam antibiotics is caused by *mecA* or *mecC* gene.

- Either gene can be situated on a mobile genetic element: the Staphylococcal Cassette Chromosome mec (SCCmec).

- The *mecC* gene occurs in small numbers in both bovine and human strains of MRSA in northern Europe.

- The most accurate way to detect methicillin resistance is to target both the orfX-SCCmec junction to detect the presence of the cassette in the *S.aureus* chromosome, and the *mecA* or *mecC* genes to ensure that the resistance gene is present.
EAT – Early Assay Termination

• What is it?
  – Real-time monitoring of reaction progress
  – Termination of the reaction when the cycle threshold of a positive reaction is crossed

• What are the benefits?
  – Positive results are reported sooner
  – For time-critical interventions valuable minutes are saved for patients that need it the most
MRSA Detected (showing Early Assay Termination)

- MRSA target DNA is 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 DNA is not detected
  - Target DNA for SCCmec is not detected and target DNA for mec (mecA/mecC) is not detected.
  - Target DNA for SCCmec is not detected and target DNA for mec (mecA/mecC) is detected.
  - Target DNA for SCCmec is detected and target DNA for mec (mecA/mecC) is not detected.
- SPC—PASS OR N/A:
  1) PASS; SPC has a Ct within the valid range and fluorescent endpoint above the threshold setting when neither mec nor SCCmec is detected;
  2) NA; if either mec or SCCmec is detected.
- Probe Check—PASS:
  All probe check results pass.
MRSA Not Detected

- MRSA DNA is not detected
  - Target DNA for SCC\textit{mec} is not detected and target DNA for \textit{mec} (\textit{mecA/mecC}) is not detected.
  - Target DNA for SCC\textit{mec} is not detected and target DNA for \textit{mec} (\textit{mecA/mecC}) is detected.
  - Target DNA for SCC\textit{mec} is detected and target DNA for \textit{mec} (\textit{mecA/mecC}) is not detected.
- SPC—PASS OR N/A:
  1) PASS; SPC has a Ct within the valid range and fluorescent endpoint above the threshold setting when neither \textit{mec} nor SCC\textit{mec} is detected;
  2) NA; if either \textit{mec} or SCC\textit{mec} is detected.
- Probe Check—PASS:
  All probe check results pass.
MRSA Not Detected

- MRSA DNA is not detected
  - Target DNA for SCC\textit{mec} is not detected and target DNA for \textit{mec} (\textit{mecA}/\textit{mecC}) is not detected.
  - Target DNA for SCC\textit{mec} is not detected and target DNA for \textit{mec} (\textit{mecA}/\textit{mecC}) is detected.
  - Target DNA for SCC\textit{mec} is detected and target DNA for \textit{mec} (\textit{mecA}/\textit{mecC}) is not detected.
- SPC—PASS OR N/A:
  1) PASS; SPC has a Ct within the valid range and fluorescent endpoint above the threshold setting when neither \textit{mec} nor SCC\textit{mec} is detected;
  2) NA; if either \textit{mec} or SCC\textit{mec} is detected.
- Probe Check—PASS:
  All probe check results pass.
Reasons to Repeat the Assay

- An INVALID result indicates that the sample was not properly processed, PCR was inhibited, or the sample was inadequate.

- An ERROR result indicates that the Probe Check Control failed or maximum pressure limits were exceeded.

- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, a load error occurred, or the software was closed prematurely.
Invalid

- Presence or absence of MRSA target DNA (mecA/mecC or SCCmec) cannot be determined. Use the instructions in the package insert Section 15 Retest Procedure, to repeat the test.

- 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 SCCmec) cannot be determined. Use the instructions in the Package Insert Section 15, Retest Procedure, to repeat the test.
- mec (mecA/mecC): NO RESULT
- SCCmec: 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 SCCmec) cannot be determined. Use the instructions in the Package Insert Section 15, Retest Procedure. 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**

- **SCCmec: 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.
<table>
<thead>
<tr>
<th></th>
<th>MRSA NxB Retest Procedure</th>
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<tbody>
<tr>
<td>1</td>
<td>Discard Used Cartridge.</td>
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</tbody>
</table>
| 2 | Remove a new cartridge and Elution Reagent vial from the package.  
  • For Dual swabs, remove the left-over swab from the transport container.  
  • For ESwab, mix the left-over Liquid Amies transport medium containing the swab sample by vortexting at high speed for 5 seconds to release the sample from the swab tip and evenly disperse in the liquid transport medium. |
| 3 | Repeat the test with a new cartridge and elution reagent. |
| 4 | Follow the Package Insert on how to run a test. |
Limitations

• Refer to the Package Insert for complete details
Technical Support

Cepheid provides technical support in the field, on the phone, by fax, and by email.

- Contact information for Cepheid offices is available on our website at http://www.cepheid.com/support.
Thank You. www.Cepheid.com