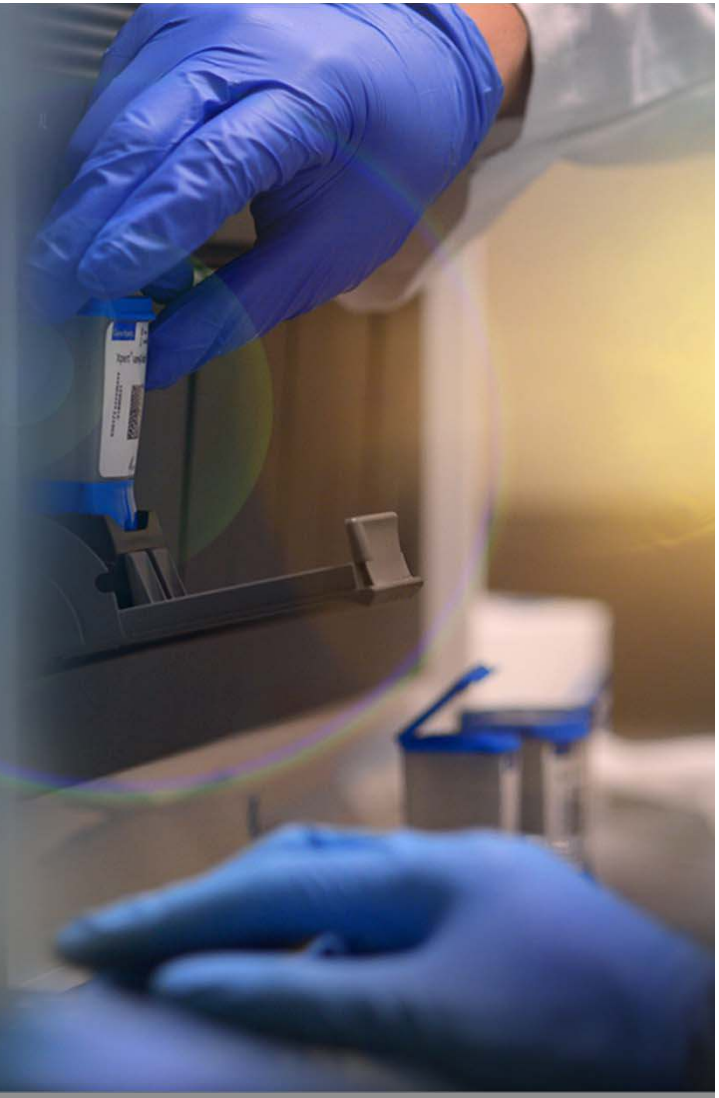
Assay Training: Xpert® MRSA NxG



Technical Training for CE-IVD product only

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301-6584 Rev. A March 2016

CE-IVD. For in-vitro diagnostic use.

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, user will be able to:

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

What is *MRSA?*

Resistance and decolonization

- Methicillin-resistant *S. aureus* (MRSA) are strains of *S. aureus* that acquired resistance to β- lactam antibiotics, 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 may include chlorhexidine body washes

S. aureus treatment:

- **Oxacillin, cephalosporine or fluoroquinolone**
- **Methicillin resistance appears ⇨ MRSA**

MRSA treatment:

- **Vancomycin or linezolid**
- **Resistance to vancomycin appears ⇨ VRSA**

[1] Investigation of Specimens for Screening for MRSA, NHS 2014

[2] Stano P. et al., in vivo 27: 873-876 (2013)

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

Staph Bacteria are a leading cause of HEALTHCARE-ASSOCIATED INFECTIONS

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a range of illnesses:

- From skin and wound infections
- Pneumonia
- Bloodstream infections
- Sepsis

S. aureus is present on the skin with a commensal carriage in 25-30% of human

Staph bacteria, including MRSA, are one of the most common causes of healthcare-associated infections

RESISTANCE OF CONCERN

Resistance to methicillin and related antibiotics and resistance to cephalosporins are of concern.

ANTIBIOTIC RESISTANCE THREATS in the United States. 2013

Köck R, et al.. Euro Surveill. 2010 Oct 14;14(41).

The Cepheid Solution

- **Detection of *mecA*, *mecC*, and SCC*mec* 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
- **Negative results in approximately 69 minutes**
  - EAT (Early Assay Termination)
- **On-demand results 24/7**
- **Random access**

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Intended Use

The Cepheid Xpert MRSA NxG Assay performed in the GeneXpert® Dx System 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) for the amplification of MRSA-specific DNA targets and fluorogenic target-specific hybridization probes for 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 nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further antimicrobial susceptibility testing.

System and Reagent Requirements

GeneXpert Systems

- GeneXpert Software Version 4.3 or higher

Test Kits (CE-IVD)

- GXMRSA-NXG-CE-10
- GXMRSA-NXG-CE-120

Materials Required but not Provided

- One of the following Collection Devices:
  - ESwab Collection and Transport System
    - (Copan #480CE or BD ESwab collection kit #220245)
    - When using the ESwab Collection kit, 300ul disposable, sterile pipet
  - 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

Xpert MRSA NxG Assay	
Catalog Number	GXMRSA-NXG-CE-10 GXMRSA-NXG-CE-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 Pouches per kit	10 or 125
Storage	2- 28 ˚C

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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.

** 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 2-28ºC. 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.**

Warnings 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 Reagent-treated 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 spent disposable pipettes

– Do not reuse spent disposable swabs

Dispose Xpert MRSA NxG Assay cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials

Cepheid Sample Collection

Dual Swab and Transport Systems

(Cepheid #900-0370 or Copan #139C LQ) STUART)

ESwab Collection and Transport System

(Copan #480CE or BD #220245)

Specimen Collection and Storage (dual swab)

Nasal Specimen Collection Protocol for use with Xpert® assays:

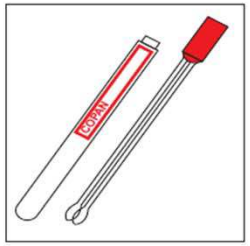
- Xpert MRSA NxG

1 Swabs to be used:

Cepheid Sample Collection Device (Part No. 900-0370

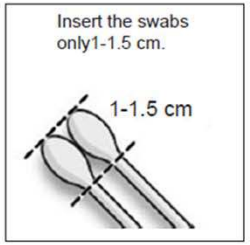
Dual Swab in Liquid Stuart Media) or the Copan Dual Swab and Transport Systems (139C LQ STUART).

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.

301-6409, Rev. A February 2016

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Specimen Collection and Storage (ESwab)

Nasal Specimen Collection Protocol for use with Xpert® assays:

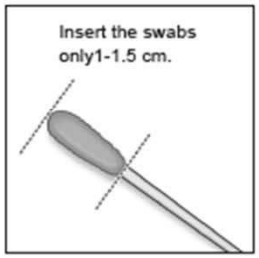
- Xpert MRSA NxG

1 Swabs to be used:

ESwab Collection and Transport System in Liquid Amies media (Copan 480CE or BD ESwab Collection Kit Part # 220245.

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.**

301-6409, Rev. A March 2018

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Xpert MRSA NxG Cartridge Preparation- dual swab

Xpert® MRSA NxG Cartridge Preparation Using dual swab

Cepheid Sample Collection Device
(Part No. 900-0370 Dual Swab in Liquid Stuart Media)
or
Copan Dual Swab and Transport Systems (Part No. 139C LQ STUART)

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

For a copy of the SDS, visit
www.cepheid.com or
www.cepheidinternational.com

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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.

301-6466, Rev A March 2013

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Xpert MRSA NxG Cartridge Preparation- ESwab

Xpert® MRSA NxG Cartridge Preparation Using an ESwab

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

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

For a copy of the SDS, visit www.cepheid.com or www.cepheidinternational.com

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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.

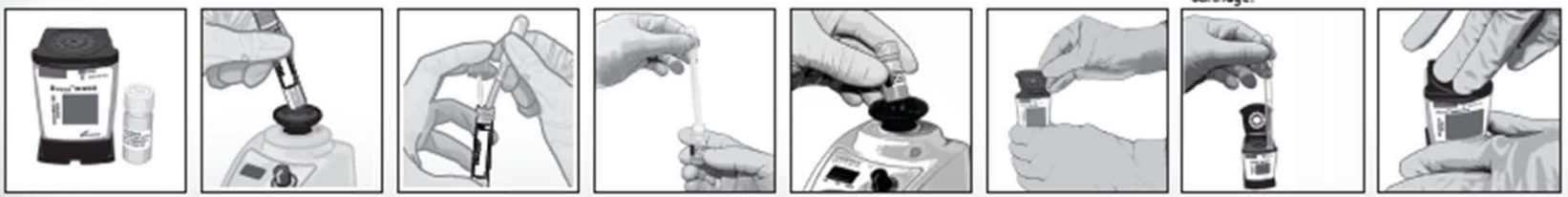
4 Recap the Elution Reagent vial and vortex at high speed for 10 seconds.

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.



301-6466, Rev. A March 2016

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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.

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Quality Control

Refer to the Package Insert for complete details

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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

Part Number	Description	Configuration	Storage
NATMRSA-6MC	MRSA Positive	0.5 mL x 6 vials	2-8˚C
NATMSSA-6MC	MSSA Positive	0.5 mL x 6 vials	2-8˚C
NATMSSE-6MC	MRSA/SA Negative	0.5 mL x 6 vials	2-8˚C
www.ZeptoMetrix.com			

1. Equilibrate to room temperature.
2. Vortex for ten seconds.
3. Dip a swab (Cepheid #900-0370 or Copan #139C LQ) into the control and transfer to the Elution Reagent vial.
4. Close vial tightly and vortex at a high speed for 10 seconds. Open the cartridge lid and transfer the entire contents of the Elution Reagent to the Sample Chamber of the cartridge. Close the cartridge lid and start the test.

Results Analysis

Refer to the Package Insert for complete details

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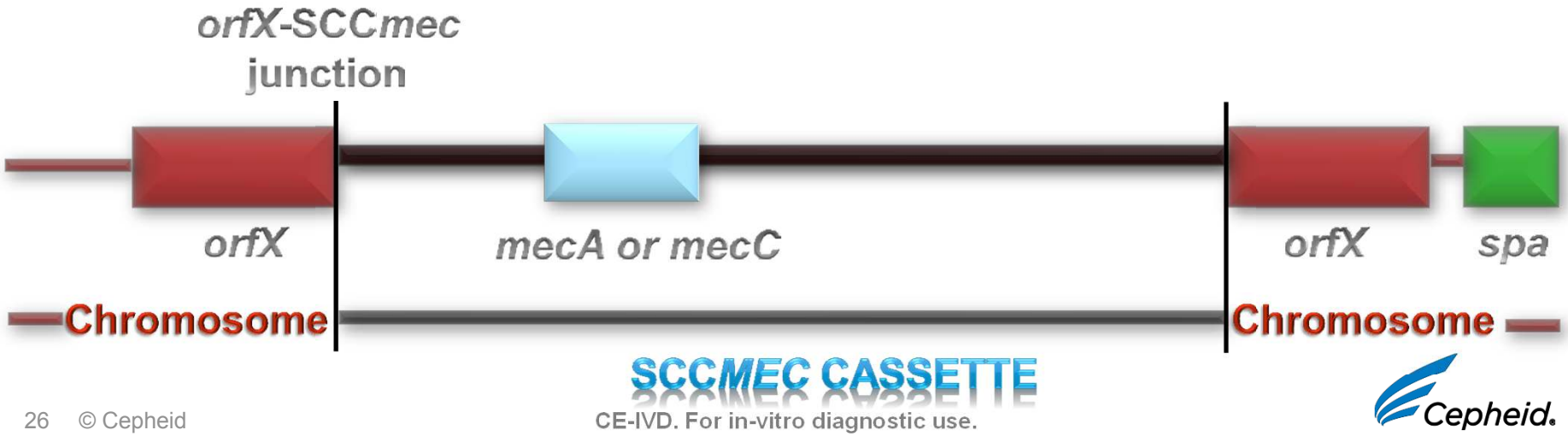
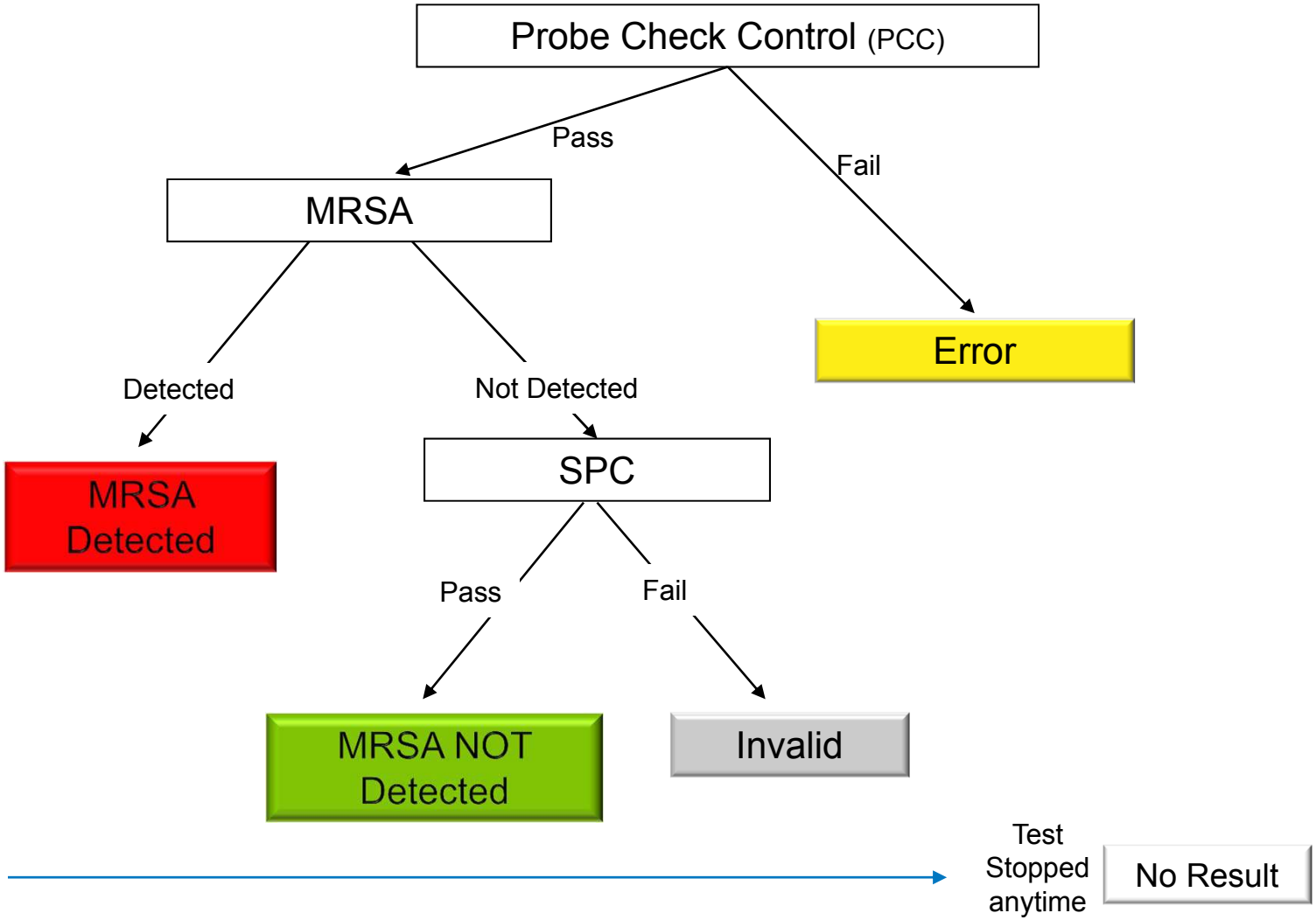
How to detect MRSA using molecular diagnostic methods

- 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 in bovine and human strains in northern Europe
- The most accurate way to detect methicillin resistant is to target both the orfX-SCCmec junction to detect the cassette, and the *mecA* or *mecC* genes to detect the resistance



Algorithm



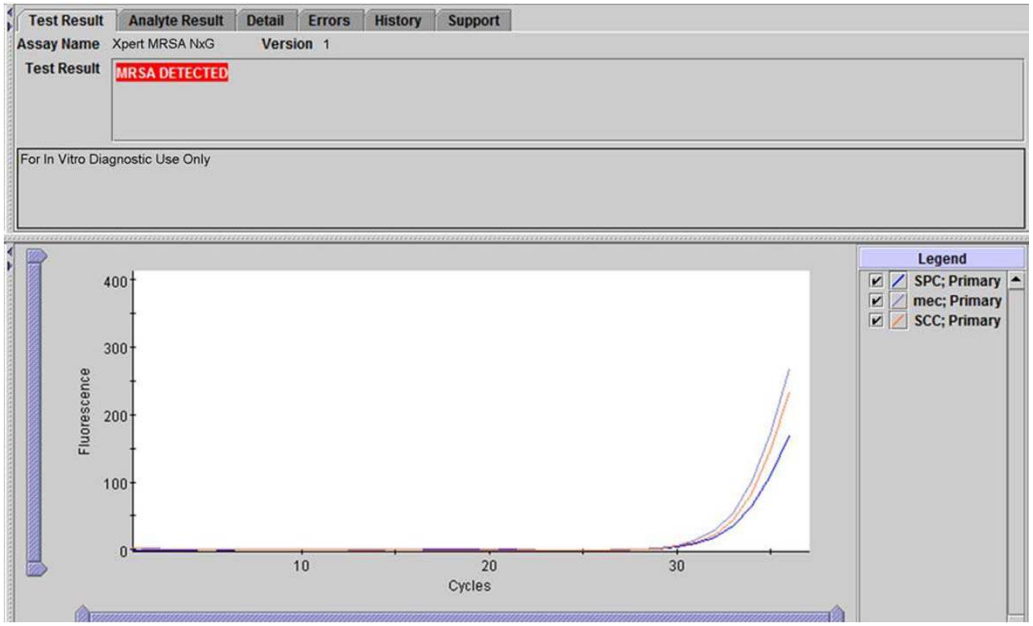
EAT – Early Assay Termination

- **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* and SCC*mec*) have a cycle threshold (Ct) within the valid range and fluorescent endpoints 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.



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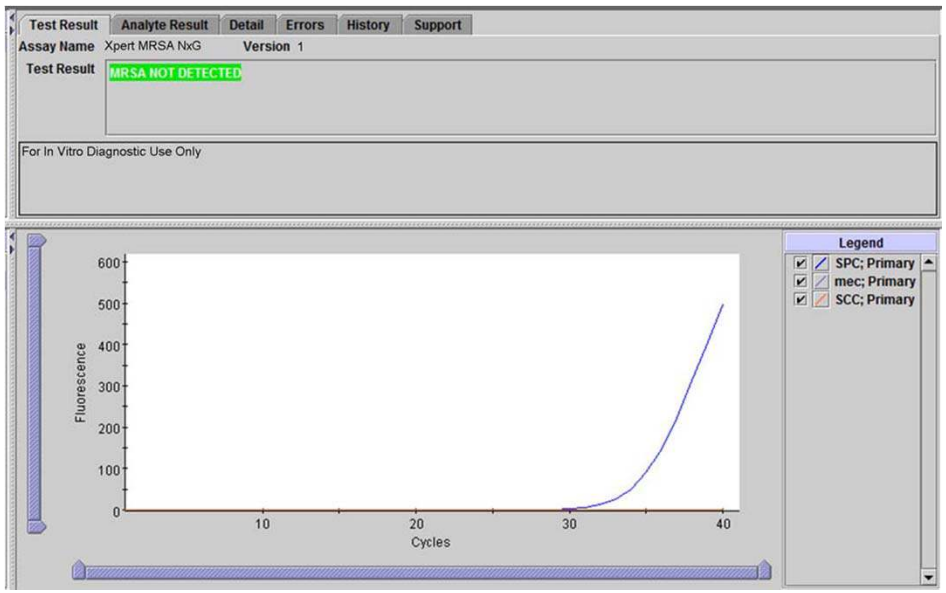
MRSA Not Detected

- MRSA target DNA sequence is not detected. SPC meets acceptance criteria.
- MRSA—NOT DETECTED:
  Target DNA for SCC*mec* is not detected, target DNA for *mec* may or may not be detected, or target DNA for SCC*mec* is detected and target DNA for *mec* 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 SCC*mec* is detected;

  2) NA; if either *mec* or SCC*mec* is detected.
- Probe Check—PASS:
  All probe check results pass.



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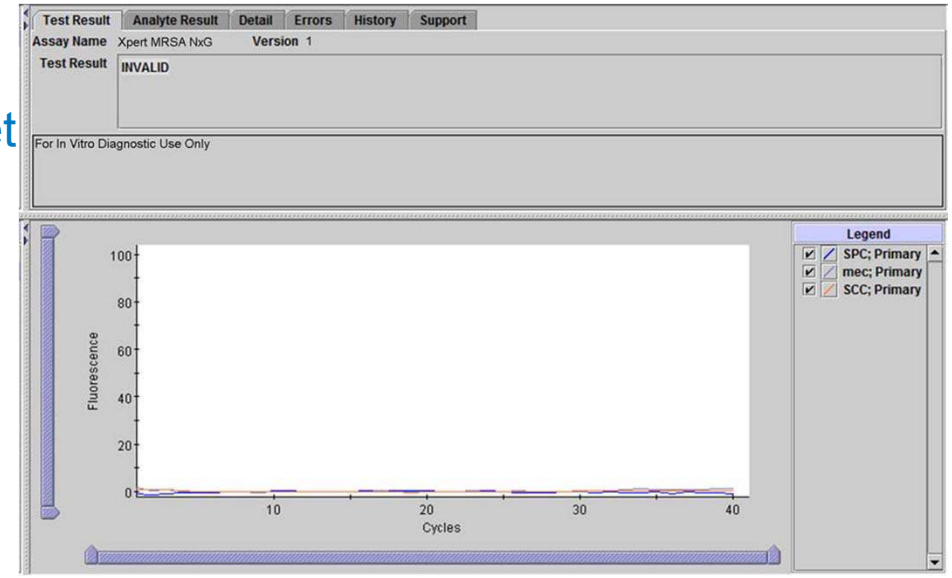
Reasons to Repeat the Assay

- **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

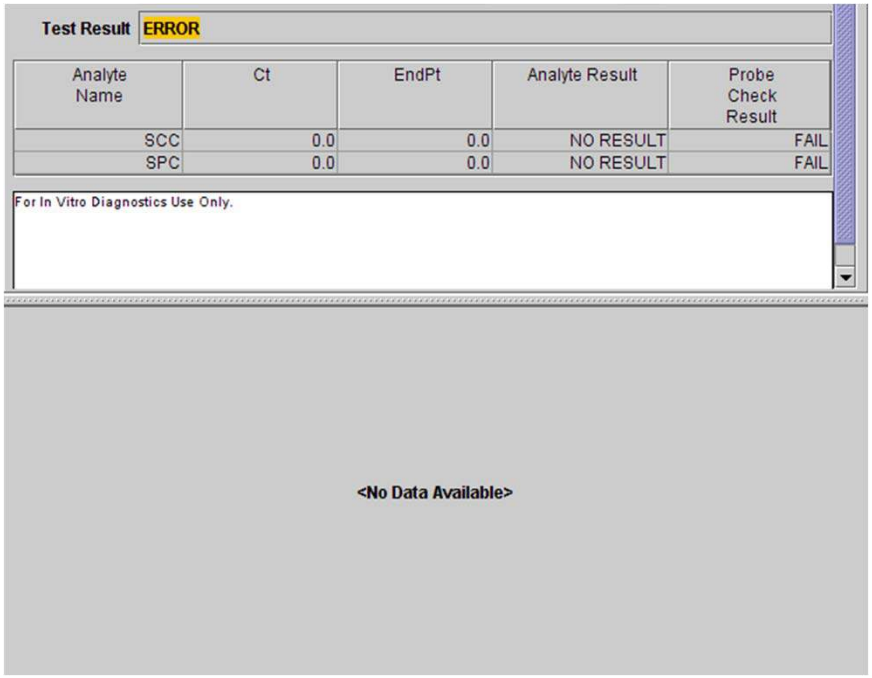
- Presence or absence of MRSA cannot be determined, repeat test with extra swab or remainder of ESwab medium. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.
- INVALID—Presence or absence of MRSA DNA cannot be determined.
- SPC—FAIL:
  SPC Ct is not within valid range and the fluorescent endpoint was below the threshold setting
- Probe Check—PASS:
  All probe check results pass.



Error

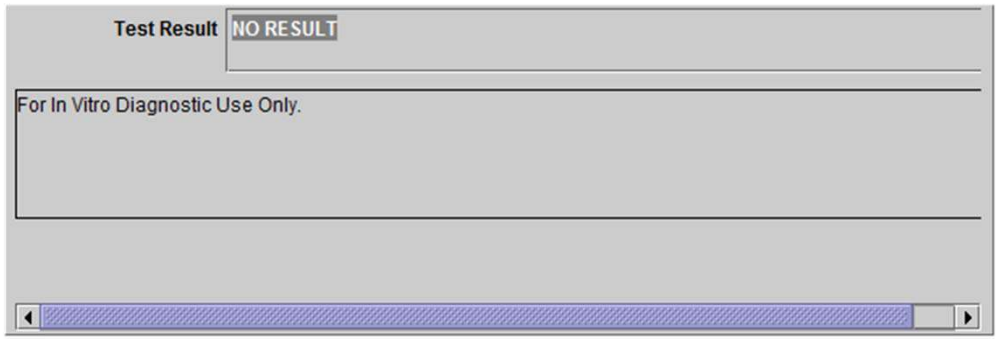
- Presence or absence of MRSA target DNA cannot be determined. Repeat test according to the instructions in the package insert.
- MRSA—NO RESULT
- SPC—NO RESULT
- Probe Check: FAIL*; all or one of the probe check results fail.

***If the Probe Check passed, the error is caused by a system component failure.**



No Result

Presence or absence of MRSA target DNA cannot be determined. Repeat test according to the instructions in the package insert.
A **NO RESULT** indicates insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.



- MRSA—NO RESULT
- SPC—NO RESULT
- Probe Check—NA (not applicable)

Retest Procedure

1	**Discard Used Cartridge.**	
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 vortexing 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.**	

Factors That Negatively Affect Results

- **Improper specimen collection**
  - Performance with other collection devices and specimen types has not been assessed.
- **Improper transport or storage of collected specimen**
  - Storage and transport conditions are specimen specific.
  - Refer to the Package Insert for the appropriate handling instructions.
- **Improper testing procedure**
  - Modification to the testing procedures may alter the performance of the test.
  - Technical error or sample mix-up can impact test results.
  - Careful compliance with the package insert is necessary to avoid erroneous results.
- **Interfering substance**
  - False negative test results or invalid results may be observed in the presence of interfering substance.
- **The number of organisms in the specimen is below the detection limit of the test**

Technical Support

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

- **Contact information for other Cepheid offices is available on our website at www.cepheid.com or www.cepheidinternational.com under the SUPPORT tab. Select the Contact Us option.**

Discussion and Q&A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