

Assay Training: Xpert[®] MRSA/SA SSTI

For US-IVD and CE-IVD product only

Cepheid Training Center



Training Agenda

- Xpert[®] MRSA/SA SSTI Training
 - Reagents
 - Sample collection
 - Kit storage and handling
 - Preparing the cartridge
 - Quality control
 - Results analysis
- Discussion



Training Xpert[®] MRSA/SA SSTI Objectives

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

- Store and handle the Xpert[®] MRSA/SA SSTI kit.
- Follow proper laboratory safety precautions.
- Collect appropriate specimen types and transport specimens appropriately.
- Perform the cartridge set up and run the assay.
- Report the various software-generated results.
- Understand the assay control strategy.

The Cepheid Solution

- Two controls for each individual sample
 - Sample Processing Control (SPC)
 - Probe Check Control (PCC)
- Simple and easy to use
- Closed cartridge system
- On-demand results 24/7
- Random access



Intended Use

The Cepheid Xpert® MRSA/SA Skin and Soft Tissue Infection Assay (Xpert® MRSA/SA SSTI Assay) performed in the GeneXpert® Dx System is a **qualitative *in vitro* diagnostic test** intended for the detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) from skin and soft tissue infection swabs. The test utilizes automated realtime polymerase chain reaction (PCR) to detect MRSA/SA DNA.

The Xpert® MRSA/SA SSTI Assay is **indicated for use in conjunction with other laboratory tests** such as microbiology culture, and clinical data available to the clinician as an aid in the detection of MRSA/SA from skin and soft tissue infections. The Xpert® MRSA/SA SSTI Assay is **not intended to monitor treatment for MRSA/SA infections. Concomitant cultures for SA and MRSA are necessary to recover organisms for susceptibility testing or epidemiological typing.** In a mixed culture containing MRSA/SA and other organisms (e.g. Gram negative bacilli, yeast), results can be false negative or variable depending on the concentration of MRSA/SA present, particularly if the concentration of MRSA/SA is close to the limit of detection (LoD) of the assay.

System and Reagent Requirements

GeneXpert Systems

- GeneXpert software version 2.1 or higher

Test Kits

- US-IVD: GXMRSA/SA-SSTI-10
- CE-IVD: GXMRSA/SA-SSTI-CE

Specimen Collection Device

- Cepheid Sample Collection Device (900-0370) or Copan equivalent

Materials Required but not Provided

- Disposable transfer pipettes
- Vortex mixer
- Sterile gauze

Optional

- Uninterruptible Power Supply/ Surge Protector
- Printer

Good Laboratory Practice

Personnel Protective Equipment (PPE)

- Wear clean lab coats and gloves
- Change gloves between processing samples

Lab Bench area

- Clean work surfaces routinely with:
 - ✓ 1:10 dilution of household bleach
 - ✓ 70% Ethanol Solution

** Final Active Chlorine concentration should be 0.5% regardless of the household bleach concentration in your country*
- After cleaning, ensure that the work surfaces are dry

Specimens, Samples, and Kits Storage

- Store specimens and sample away from kit to prevent contamination

Equipment

- Use filtered tips when recommended
- Follow the manufacturer's requirements for calibration and maintenance of equipment

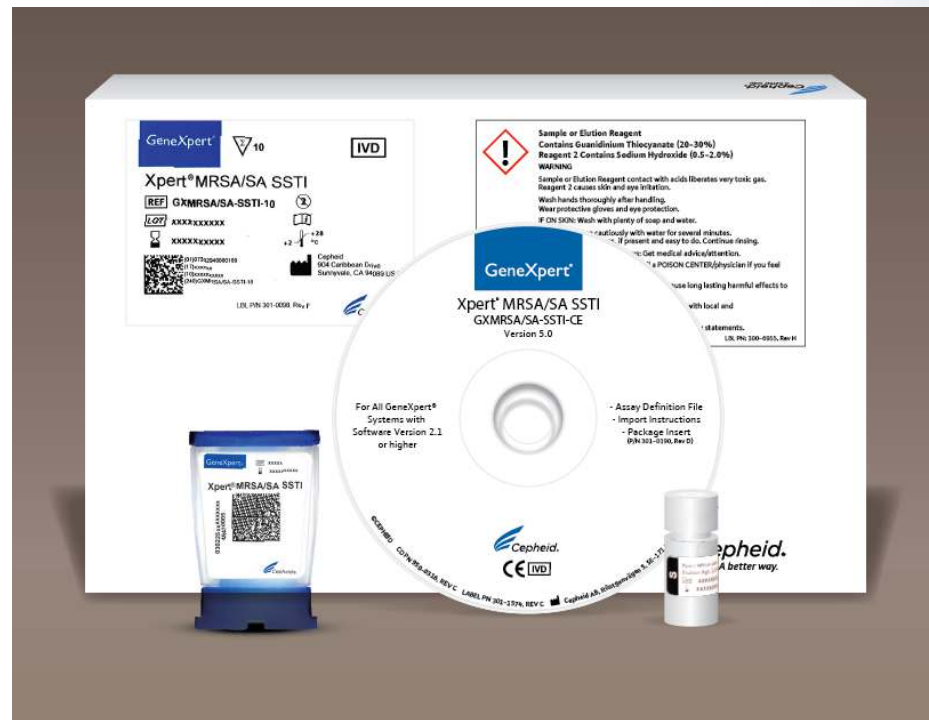
Kit Handling



Xpert[®] MRSA/SA SSTI Kit Contents

Xpert[®] MRSA/SA SSTI Assay

Catalog Number	GXMRSA/SA-SSTI-10, GXMRSA/SA-SSTI-CE
Tests Per Kit	10
Cartridge Contents	Reagent beads
	Liquid Reagents
Kit CD	Assay Definition File (ADF)
	Assay Import Instructions
	Package Insert (PDF)
Elution Reagent pouches per kit	10 x 2.0 mL
Storage	2- 28 °C



Xpert[®] MRSA/SA SSTI Storage and Handling

- Store the Xpert[®] MRSA/SA SSTI cartridges and reagents at 2–28°C
- Follow your institution's safety procedures for working with chemicals and handling biological samples
- Do not use collection devices that have not been validated by Cepheid
- Open the cartridge lid only when adding the sample, close the lid and proceed with processing

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 you have added the sample
 - that has a damaged reaction tube
 - that has been used; each cartridge is single-use to process one test
 - that is expired
- Do not reuse pipettes
- Do not reuse swabs



Dispose Xpert® MRSA/SA SSTI cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials.

Specimen Collection



Cepheid Sample Collection Device



Use the Cepheid Sample Collection Device (P/N 900-0370) or Copan equivalent.

Swab specimens of skin and soft tissue infections can be taken following the user institution's standard procedures.

Xpert[®] MRSA/SA SSTI Specimen Transport and Storage

Specimen	Transport and Storage Temperature (°C)	Storage Time
Skin and soft tissue infection swabs	2-8 °C	5 days
	Room Temperature	24 hours

MRSA/SA SSTI Cartridge Preparation

Xpert Cartridge Preparation

- Xpert MRSA
- Xpert MRSA/SA SSTI
- Xpert vanA
- Xpert C. difficile
- Xpert C. difficile/Epi
- Xpert SA Nasal Complete
- Xpert Norovirus

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

For a copy of the SDS, visit www.cephheid.com or www.cephheidinternational.com

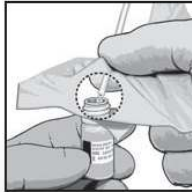
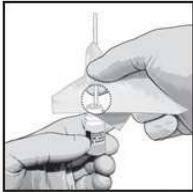
Cepheid Technical Support

US office
(888) 838-3222, Option 2
techsupport@cephheid.com

European office
+33 563 82 53 19
support@cephheidurope.com



- 1 Obtain one Xpert cartridge and one Sample Reagent vial for each sample.
- 2 Insert the swab into the Sample Reagent vial.
- 3 Break the swab at the score mark near the mouth of the vial.
- 4 Recap the Sample Reagent vial and vortex for 10 seconds.
- 5 Open the Xpert cartridge lid.
- 6 Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.
- 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.

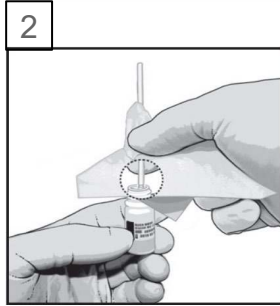


Note: Do not hold the swab below the score mark. Use gauze or its equivalent to minimize the risk of contamination.

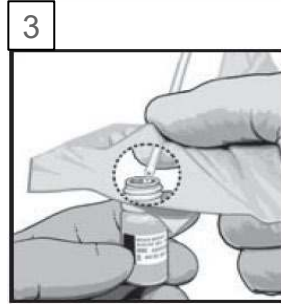
MRSA/SA SSTI Cartridge Preparation



Obtain one Xpert cartridge and one Sample Reagent vial for each sample.



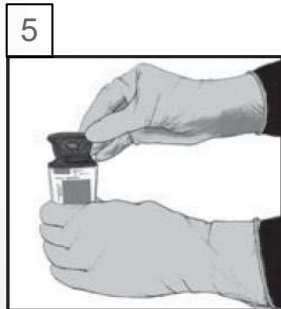
Insert the swab into the Sample Reagent vial.



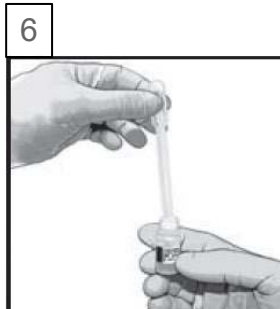
Break the swab at the score mark near the mouth of the vial.



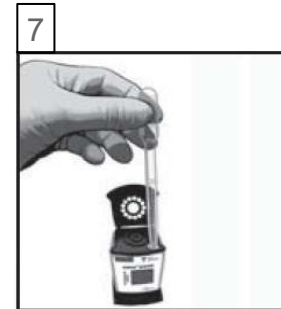
Recap the Sample Reagent vial and vortex for 10 seconds.



Open the Xpert cartridge lid.



Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.



Empty the pipette into the sample chamber.



Close the Xpert cartridge lid.

9
Start the test within the timeframe specified in the package insert.

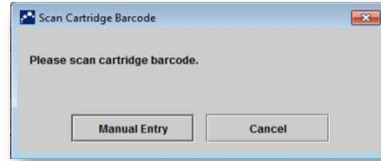
Run a Test

1 Create Test



Start the test within **15 minutes** after adding the sample to the cartridge

2 Scan barcodes: Cartridge/ Patient and/or Sample ID



*By default, do not click on **Manual Entry** or **Cancel***

3 Scan the cartridge



"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx

Create a Test on GeneXpert Dx Software

4 Complete the fields as required

5 The Assay Protocol is selected automatically

6 The module is selected automatically

7 Click on Start Test

8 A green light will flash on the module
Load the cartridge into module and close the door

The screenshot shows the 'Create Test' software interface. It contains several input fields and buttons. An orange box highlights the 'Patient ID', 'Sample ID', 'Patient ID 2', and 'Last Name' fields. Another orange box highlights the 'Select Assay' dropdown menu, which is currently set to 'Xpert Assay name'. A third orange box highlights the 'Select Module' dropdown menu, which is set to 'A3'. A fourth orange box highlights the 'Start Test' button. A mouse cursor is pointing at the 'Start Test' button. Arrows from the numbered instructions point to these elements: instruction 4 points to the patient ID fields, instruction 5 points to the 'Select Assay' dropdown, instruction 6 points to the 'Select Module' dropdown, and instruction 7 points to the 'Start Test' button.



Automated Xpert[®] Protocol



Waste Disposal

- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of standard precautions.
- Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.
- If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

Quality Control

*Refer to the Package Insert for
complete details*



Cepheid Assay Control Strategy

Xpert® MRSA/SA-SSTI Quality Controls

- Each Xpert cartridge is a self-contained test device.
- Cepheid designed specific molecular methods that include internal controls that enable the system to detect specific failure modes within each cartridge.
 - Probe Check Control (PCC)
 - Sample Processing Control (SPC)

Internal Quality Controls

Probe Check Controls (PCC)

- Before the PCR step, fluorescence signal is measured on all probes and compared with default factory settings to monitor
 - bead rehydration
 - reaction tube filling
 - probe integrity
 - dye stability

Sample Processing Controls (SPC)

- Verifies that conditions for adequate sample processing were met
- Detects PCR inhibition
- Should be positive in a negative sample
- Can be positive or negative in a positive sample

Results Analysis

*Refer to the Package Insert for
complete details*



Results Summary

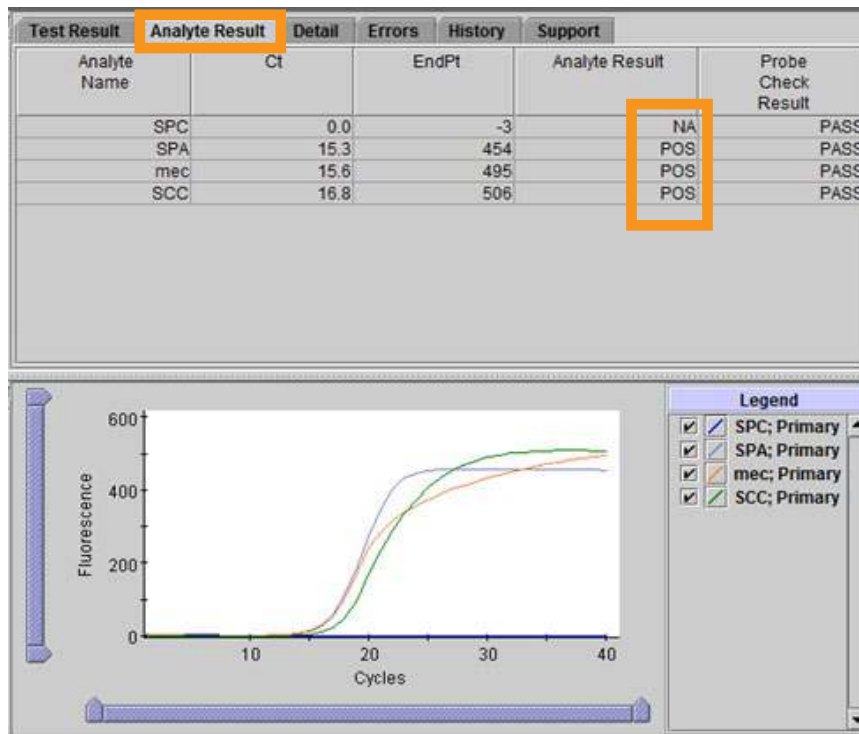
Result displayed	SPA	mec	SCC	SPC
MRSA POSITIVE	+	+	+	+/-
SA POSITIVE				
MRSA NEGATIVE	+	-	+	+/-
SA POSITIVE		+	-	
		-	-	
MRSA NEGATIVE	-	+/-	+/-	+
SA NEGATIVE				
INVALID	-	-	-	-
ERROR	NO RESULT	NO RESULT	NO RESULT	NO RESULT
No Result	NO RESULT	NO RESULT	NO RESULT	NO RESULT

MRSA Positive/SA Positive

Test Result **MRSA POSITIVE:
SA POSITIVE**

MRSA target DNA sequences are detected/SA target DNA sequence is detected.

- MRSA POSITIVE: All MRSA targets (*spa*, *mecA*, *SCC mec*) have a valid Ct.
- SA POSITIVE: The SA target (*spa*) has a valid Ct.
- SPC: NA (not applicable); SPC is ignored because MRSA amplification may compete with this control.
- Probe Check: PASS
All probe check results pass.



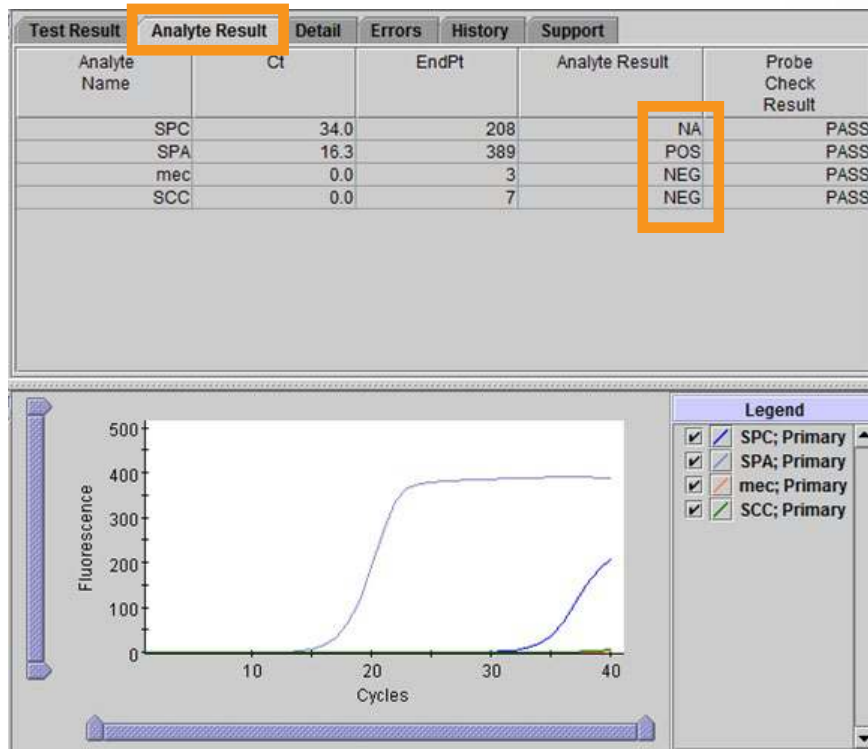
MRSA Negative/SA Positive

Test Result **MRSA NEGATIVE**
SA POSITIVE

MRSA target DNA sequences are not detected/SA target DNA sequence is detected.

- SA POSITIVE: The SA target (*spa*) has a valid Ct. Target DNA for *SCCmec* is not detected, target DNA for *mecA* may or may not be detected, or target DNA for *SCCmec* is detected and target DNA for *mecA* is not detected
- SPC: NA (not applicable)
SPC is ignored because SA amplification can 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 SA.

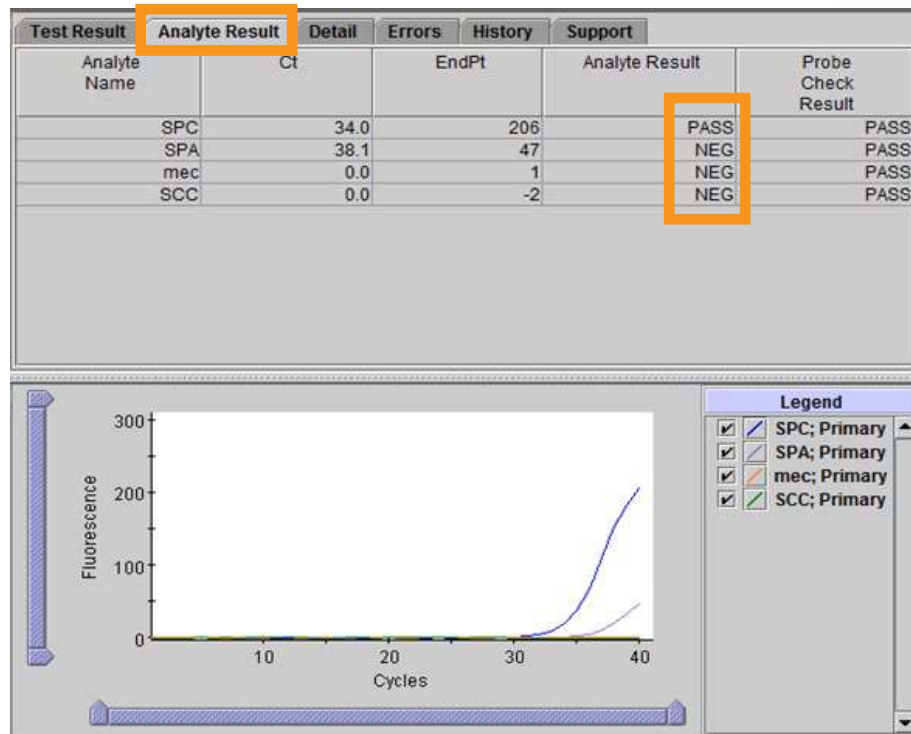


MRSA Negative/SA Negative

Test Result **MRSA NEGATIVE;
SA NEGATIVE**

Staphylococcus aureus target DNA sequence is not detected. SPC meets acceptance criteria.

- **NEGATIVE:** *Staphylococcus aureus* target (*spa*) DNA is not detected. Target DNA for *mecA* may or may not be detected, or target DNA for *SCCmec* may or may not be detected
- SPC: PASS; SPC has a valid Ct.
- Probe Check: PASS
All probe check results pass.



Troubleshooting



Factors That Negatively Affect Results

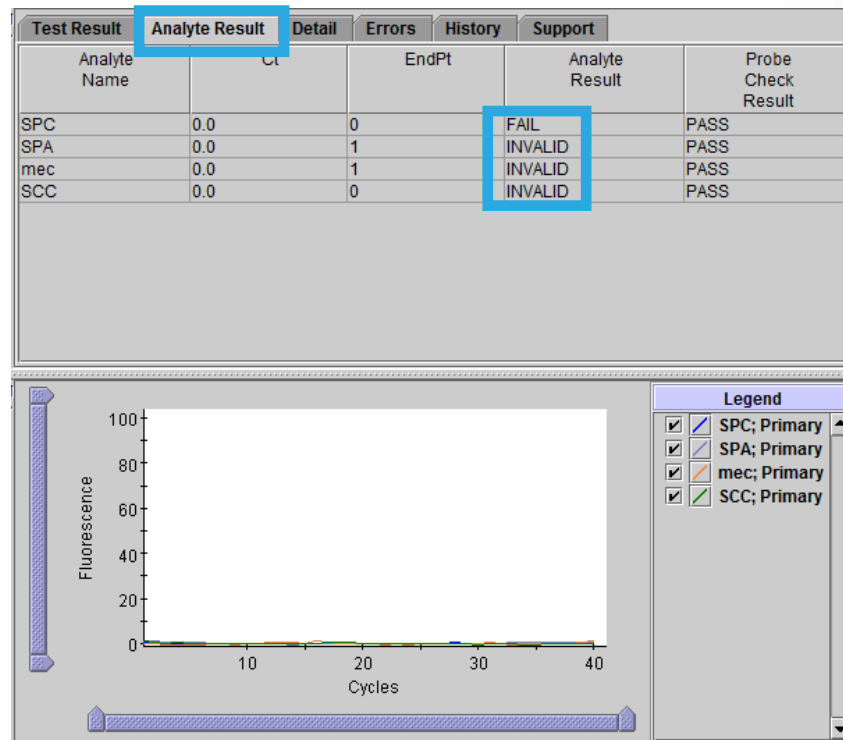
- Improper specimen collection
 - The bacterial load in the specimen is below the detection limit of the test
 - Performance with other specimen types has not been assessed
- Improper transport or storage of collected specimen
 - Storage and transport conditions are specimen specific
 - Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
 - Modification to the testing procedures may alter the performance of the test
 - Careful compliance with the package insert is necessary to avoid erroneous results

INVALID

Test Result INVALID

Presence or absence of MRSA/SA target DNA sequences cannot be determined. SPC does not meet the acceptance criteria, the sample was not correctly processed, or PCR was inhibited.

- INVALID: Presence or absence of *Staphylococcus aureus* DNA cannot be determined.
- SPC– FAIL: SPC target result is negative, and the SPC Ct is not valid.
- Probe Check: PASS
All probe check results pass.



ERROR

Test Result **ERROR**

Presence or absence of MRSA/SA target DNA sequences cannot be determined.

- MRSA: NO RESULT
- SA: NO RESULT
- SPC: NO RESULT
- Probe Check: FAIL*
One or more of the probe check results failed.

*If the probe check passed, a system component failed.

#	Description	Detail	Time
1	Post-run analysis error	Error 5007: SCC) probe check failed. Probe check value of 0 for reading number 2 was below the minimum of 33	01/25/15 05:07:22
2	Post-run analysis error	Error 5007: [SPC) probe check failed. Probe check value of 0 for reading number 2 was below the minimum of 222	01/25/15 05:07:22

NO RESULT

Test Result **NO RESULT**

Presence or absence of MRSA/SA target DNA sequences cannot be determined. Insufficient data were collected to produce a test result.

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

Test Result				
Analyte Name	Analyte Result	Ct	EndPt	Probe Check Result
SPC	0.0	0	NO RESULT	NA
SPA	0.0	0	NO RESULT	NA
mec	0.0	0	NO RESULT	NA
SCC	0.0	0	NO RESULT	NA

Retest Procedure

Xpert Retest Procedure

- Xpert C. difficile
- Xpert C. difficile/Epi
- Xpert MRSA/SA SSTI
- Xpert vanA

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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techsupport@cepheid.com

European office
+33 563 82 53 19
support@cepheid europe.com

Follow this retest procedure within 3 hours of an **ERROR, INVALID, or NO RESULT**.

Otherwise:

Retest with the remaining specimen if the volume is sufficient

or

Collect a new specimen and process the sample per the package insert.



- 1 Retain the used cartridge. Obtain a new Xpert cartridge and a new Sample Reagent vial.



- 2 Transfer all the remaining contents from the Sample chamber of the used cartridge to a new Sample Reagent vial.



- 3 Recap the Sample Reagent vial and vortex for 10 seconds.



- 4 Open the new Xpert cartridge lid.



- 5 Aspirate all of the Sample Reagent vial contents with a disposable transfer pipette.



- 6 Empty the pipette into the sample chamber.



- 7 Close the Xpert cartridge lid and start the test within the timeframe specified in the package insert.



Interfering Substances

The following substances were shown to interfere with the quantification of the MRSA/SA SSTI Assay or impact the assay specificity:

Substance	Concentration
StaphA ⁺ Septic	5% w/v
Hydrocortisone	5% w/v
Antibacterial hand sanitizer	5% w/v

Please refer to Xpert[®] MRSA/SA SSTI Package Insert for data on interfering substances.

Technical Assistance

- Before contacting Cepheid Technical Support, collect the following information:
 - Product name
 - Lot number
 - Serial number of the System
 - Error messages (if any)
 - Software version and, if applicable, Computer Service Tag number
- Log your complaint online using the following link <http://www.cepheid.com/us/support> :Create a Support Case

Region	Telephone	Technical Support Email
US	+ 1 888 838 3222	techsupport@cepheid.com
Australia and New Zealand	+ 1800 130 821	techsupportANZ@cepheid.com
Brazil and Latin America	+ 55 11 3524 8373	latamsupport@cepheid.com
China	+ 86 021 5406 5387	techsupportchina@cepheid.com
France	+ 33 563 825 319	support@cepheideurope.com
Germany	+ 49 69 710 480 480	support@cepheideurope.com
India, Bangladesh, Bhutan, Nepal, and Sri Lanka	+ 91 11 48353010	techsupportindia@cepheid.com
Italy	+ 39 800 902 567	support@cepheideurope.com
Japan	+ 0120 95 4886	support@japan.cepheid.com
South Africa	+ 27 861 22 76 35	support@cepheideurope.com
United Kingdom	+ 44 3303 332 533	support@cepheideurope.com
Belgium and Netherlands	+33 563 825 3319	support@cepheideurope.com
Other European, Middle East, and African countries	+ 33 563 825 319 + 971 4 253 3218	support@cepheideurope.com



Thank You.



www.Cepheid.com