Assay Training: Xpert® MRSA/SA Blood Culture

For US-IVD and CE-IVD product only

Cepheid Training Center

CE IVD In Vitro Diagnostic Medical Device

301-2327, Rev. B November 2017
US-IVD and CE-IVD. For *in vitro* diagnostic use.

Training Agenda

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

Training Objectives

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

- Store and handle the Xpert MRSA/SA BC 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

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Intended Use

The Cepheid® Xpert MRSA/SA Blood Culture Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test intended for the detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) DNA directly from positive blood cultures. The assay utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA/SA specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed directly on positive blood culture specimens from BD BACTEC™ Plus Aerobic/F, BacT/ALERT® SA (Standard Aerobic), or VersaTREK REDOX 1® (aerobic) blood culture bottles that are determined by Gram Stain as gram-positive cocci in clusters (GPCC) or as gram-positive cocci in singles (GPC).

The Xpert MRSA/SA Blood Culture Assay is indicated for use in conjunction with other laboratory tests, such as culture, and clinical data available to the clinician as an aid in the detection of MRSA/SA from positive blood cultures. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing or for epidemiological typing. The Cepheid Xpert MRSA/SA Blood Culture Assay is not intended to monitor treatment for MRSA/SA infections.

System and Reagent Requirements

GeneXpert Systems

- GeneXpert software version 4.0 or higher

Test Kits

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

Materials Required but not Provided

- Disposable transfer pipettes
- Vortex mixer

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 Blood Culture Kit Contents

Xpert MRSA/SA BC Assay	
Catalog Number	GXMRSA/SA-BC-10, GXMRSA/SA-BC-CE-10
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
Disposable Transfer Pipettes	12
Storage	2- 28 ˚C

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Xpert MRSA/SA BC Storage and Handling

- Store the Xpert MRSA/SA BC 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 BC cartridges and reagents according to your institution's and country's guidelines for disposal of hazardous materials.

Specimen Collection

Specimen Collection, Transport, and Storage

Specimen	Transport and Storage Temperature (˚C)	Storage Time
Positive Blood Culture Bottles that show gram-positive cocci in clusters (GPCC) or gram-positive cocci in singles (GPC) by gram stain	2-8 ˚C	3 days
	Room Temperature	24 hours

Validated blood culture bottles include:
BD BACTEC™ Plus Aerobic/F
BacT/ALERT® SA (Standard Aerobic)
VersaTREK REDOX 1® (aerobic) blood culture bottles

MRSA/SA BC Cartridge Preparation

Xpert® MRSA/SA Blood Culture Cartridge Preparation

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.

Cepheid Technical Support
US office
(888) 838-3222, Option 2
techsupport@cepheid.com

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

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A better way.

1 Obtain one cartridge and one Elution Reagent vial.

2 After gently mixing sample by hand, use the provided 50 µL transfer pipette to dispense the sample into the Elution Reagent vial. Some residue may remain in the pipette.

3 Close the Elution Reagent cap and vortex at high speed for 10 seconds.

4 Open the cartridge lid.

5 Using a clean transfer pipette (not supplied), transfer the entire contents of the Elution Reagent vial to the sample chamber of the cartridge.

6 Close the cartridge lid.

7 Insert the cartridge and start the assay within the timeframe specified in the package insert.

301-0071, Rev. B July 2013

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MRSA/SA BC Cartridge Preparation

1

Obtain one Xpert cartridge and one Elution Reagent vial.

2

After gently mixing sample by hand, use the provided 50 µL transfer pipette to dispense the sample into the Elution Reagent vial. Some residue may remain in the pipette.

3

Close the Elution Reagent cap and vortex at high speed for 10 seconds.

4

Open the Xpert cartridge lid.

5

Using a clean transfer pipette (not supplied), transfer the entire contents of the Elution Reagent vial to the sample chamber of the cartridge.

6

Close the Xpert cartridge lid.

7

Insert the cartridge and start the assay within the timeframe specified in the package insert.

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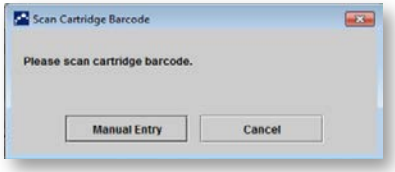
Run a Test

1 Create Test

GeneXpert

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

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

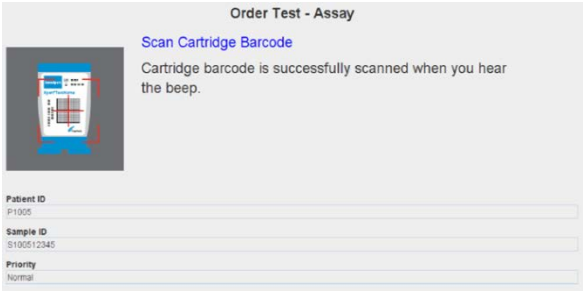


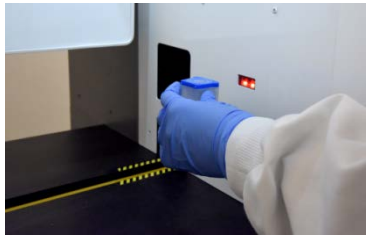
By default, do not click on **Manual Entry** or **Cancel**

3 Scan the cartridge

GeneXpert Infinity

Place the cartridge on the conveyor within 15 minutes of adding the sample.





"For complete details on how to run a test, refer to the Package Insert and the GeneXpert Dx or Xpertise Operator Manuals.

Create a Test on GeneXpert Dx Software

4 Complete the fields as required

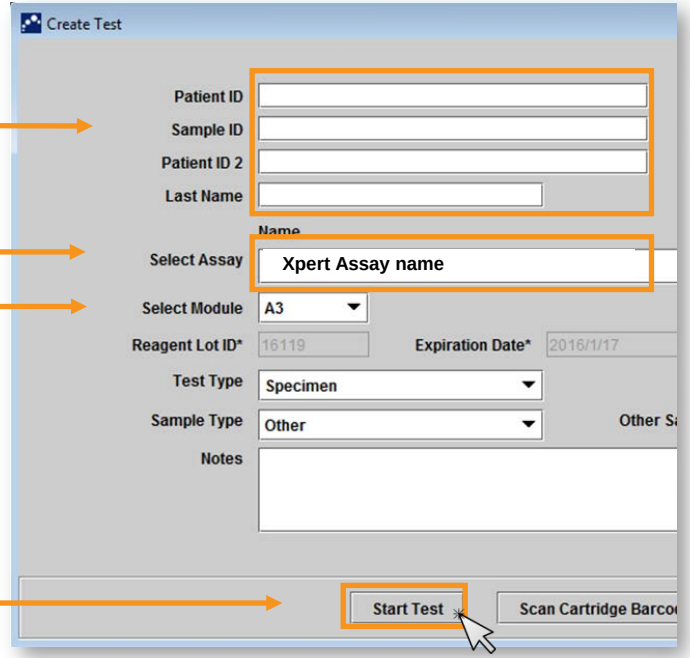
5 The Assay Protocol is selected automatically

6 The module is selected automatically

DO NOT CHANGE IT!!!

7 Click on Start Test

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

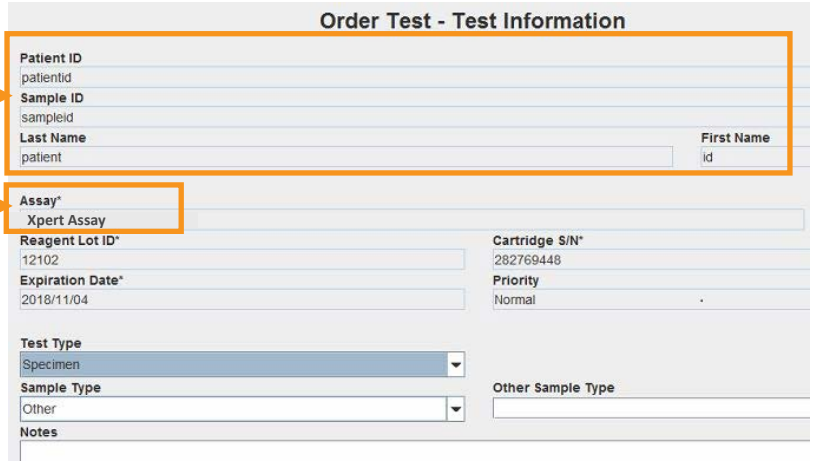


Create a Test on Xpertise Software

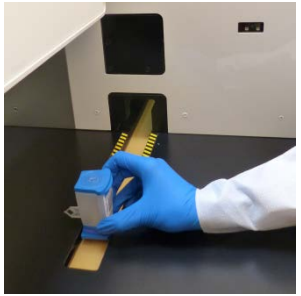
4 Complete the fields as required

5 The Assay Name Protocol is selected automatically

6 Click on SUBMIT



7 Place the cartridge into the conveyor belt



Automated Xpert MRSA/SA BC 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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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 BC 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

Commercially Available External Controls

Company	Description	Catalog Number
ZeptoMetrix http://www.zeptometrix.com	NATtrol MRSA Positive Control	NATMRSA-6MC
	NATtrol SA Positive Control	NATMSSA-6MC
	NATtrol MRSA/SA Negative Control	NATMSSE-6MC
MicroBiologics http://www.microbiologics.com	ATCC 700699 MRSA Positive Control	0158 MRSA
	ATCC 25923 SA Positive Control	0360 MSSA
	ATCC 12228 Negative Control	0371 MSSE

External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

Results Analysis

Refer to the Package Insert for complete details

Results Summary

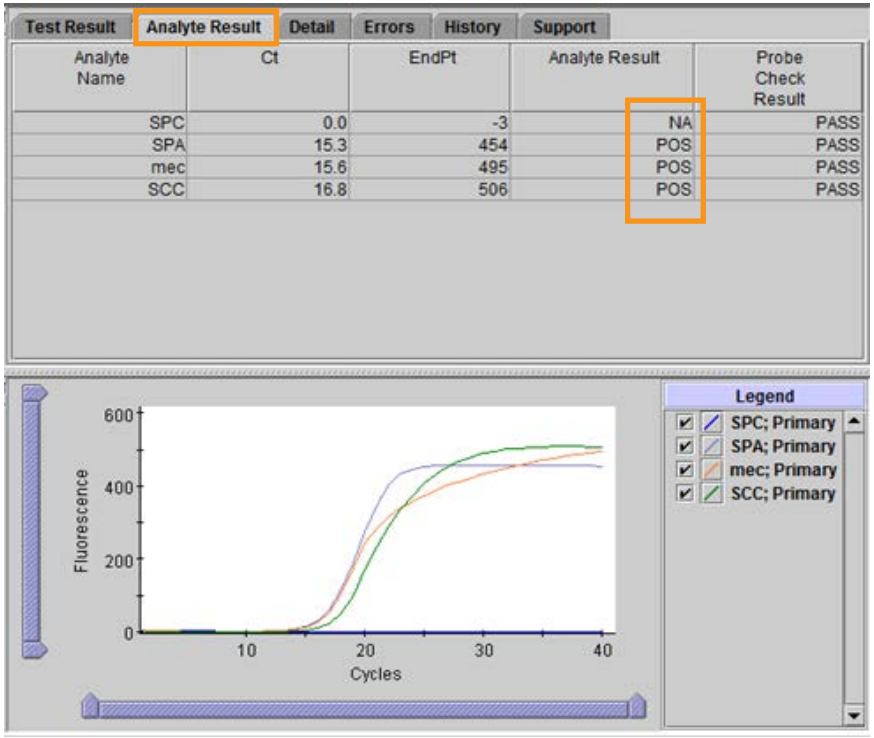
Result displayed	SPA	mec	SCC	SPC
MRSA POSITIVE SA POSITIVE	+	+	+	+/-
MRSA NEGATIVE SA POSITIVE	+	- + -	+ - -	+/-
MRSA NEGATIVE SA NEGATIVE	-	+ -	- +	+/-
INVALID	-	-	-	-
ERROR	NO RESULT	NO RESULT	NO RESULT	NO RESULT
No Result	NO RESULT	NO RESULT	NO RESULT	NO RESULT

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.

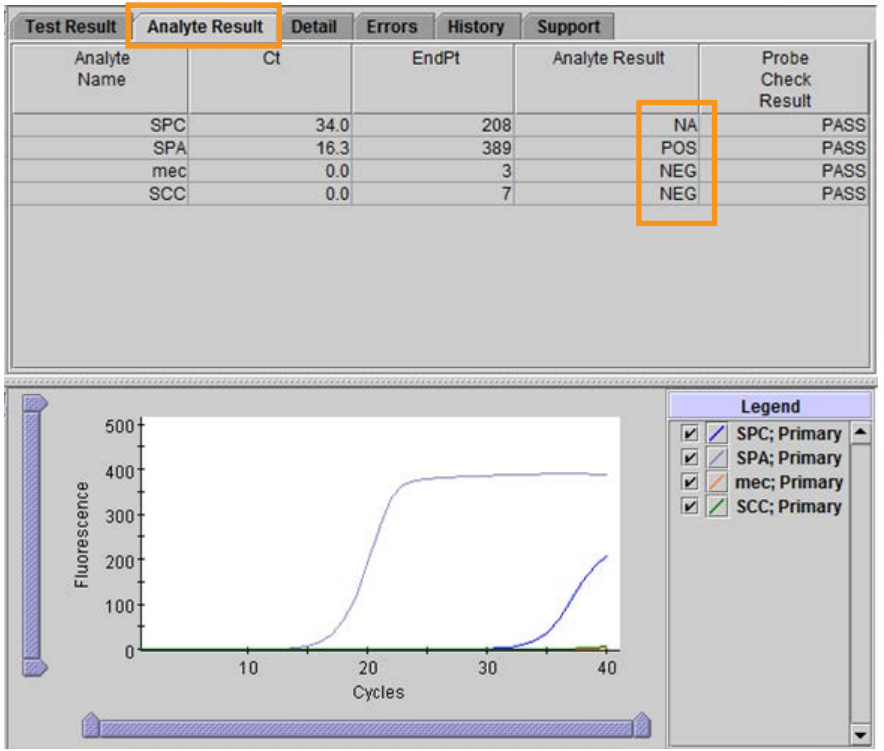


MRSA Negative/SA Positive

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.



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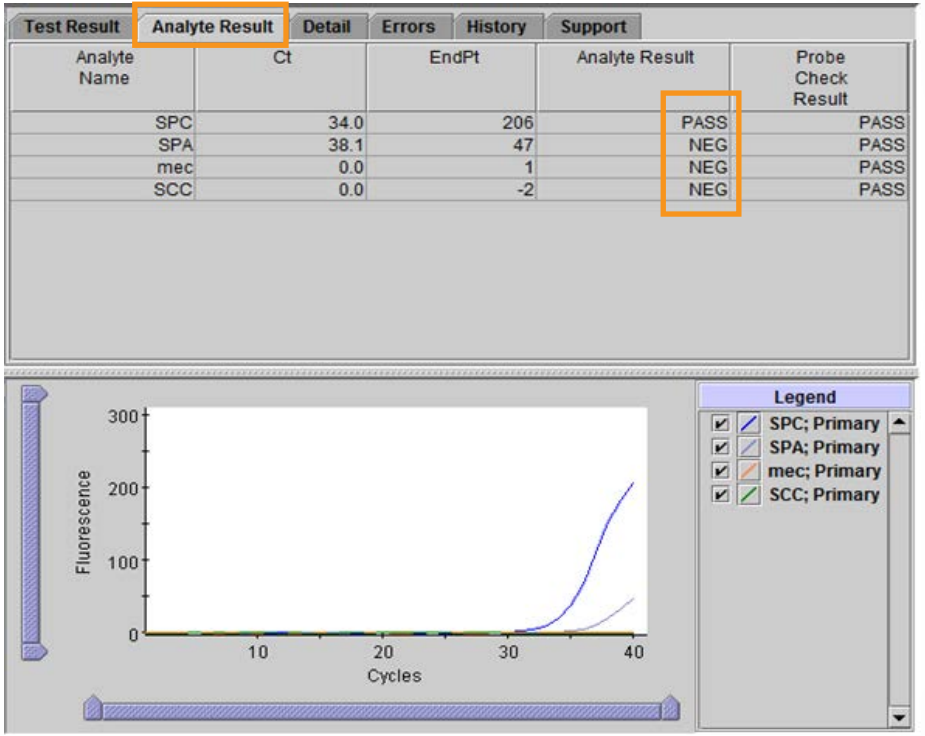
MRSA Negative/SA Negative

Test Result: MRSA NEGATIVE; SA NEGATIVE

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

- NEGATIVE: *Staphylococcus aureus* target 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

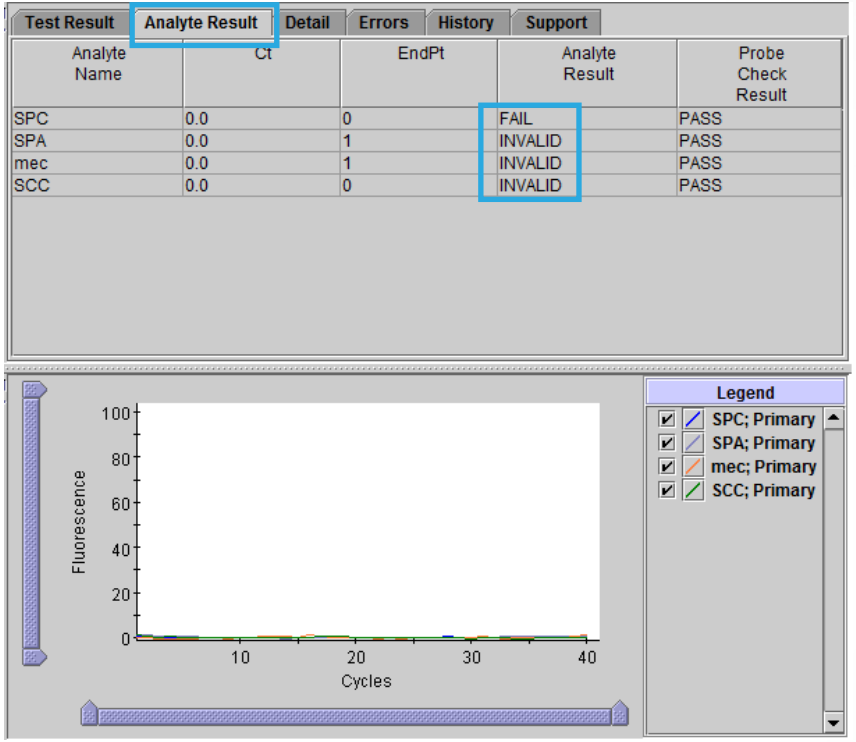
- Improper specimen collection
  - The bacterial load in the specimen is below the detection limit of the test
  - Performance with other specimen types has not been assessed
- Improper transport or storage of collected specimen
  - Storage and transport conditions are specimen specific
  - Refer to 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.



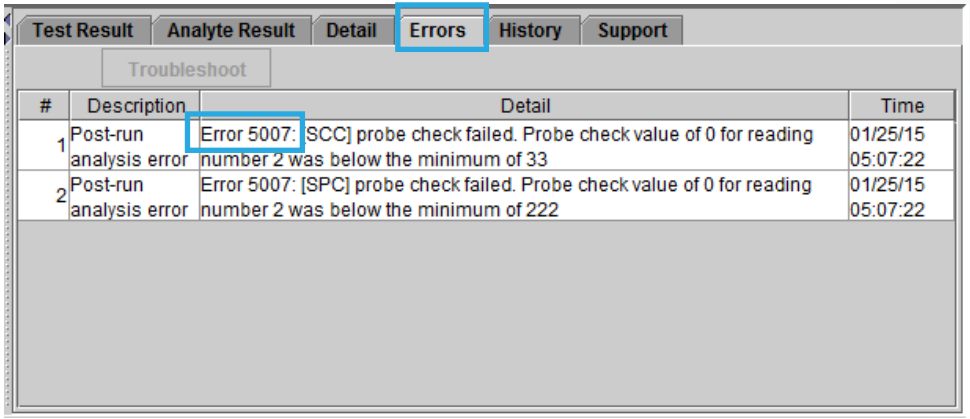
ERROR

Test Result ERROR

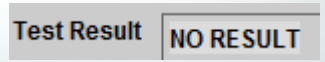
Presence or absence of MRSA/SA target DNA sequences cannot be determined.

- 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.

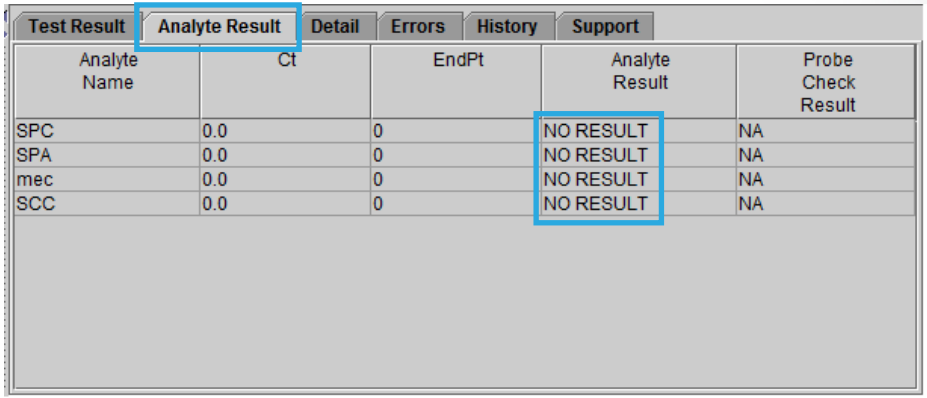


NO RESULT



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)



Analyte Name	Ct	EndPt	Analyte Result	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

MRSA/SA BC Retest Procedure

1

Discard used cartridge.

Follow your institution's safety guidelines for disposal of cartridges.

2

Repeat with a new aliquot from the blood culture bottle, or if the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample.

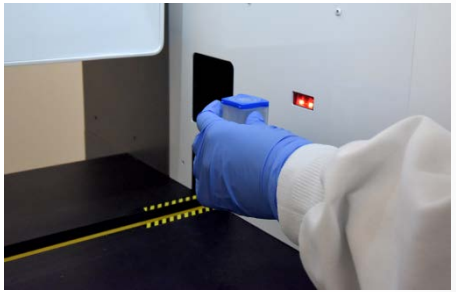
3

Obtain a new cartridge and elution vial.

Process the sample per the package insert.

4

Run the test on the system.



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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 107 884 (AU) + 0800 001 028 (NZ)	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

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