

Assay Training: Xpert[®] MRSA/SA Blood Culture

For CE-IVD product only



Training Agenda

- **Xpert® MRSA/SA Blood Culture**
 - Reagents
 - Sample collection
 - Kit storage and handling
 - Preparing the cartridge
 - Quality Controls
 - Results analysis
- **Discussion**



Training Objectives

- **At the end of the training, users will be able to:**
 - Properly store and handle the Xpert[®] MRSA/SA BC cartridge kit
 - Follow proper laboratory safety precautions
 - Collect and store appropriate specimen(s)
 - Prepare a cartridge and run the Xpert[®] MRSA/SA BC assay
 - Report the various software generated results
 - Understand the Xpert[®] MRSA/SA BC control strategy

The Cepheid Solution



- Simultaneous detection of:
 - *Staphylococcus aureus* (SA)
 - Methicillin-resistant *Staphylococcus aureus* (MRSA)
- On-board internal controls for each sample
 - Probe Check Control (PCC)
 - Sample Processing Control (SPC)
- Results in approximately 60 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results
- Random access

Intended Use

- The Cepheid Xpert® MRSA/SA Blood Culture test, performed on the GeneXpert® Instrument Systems, is a **qualitative *in vitro* diagnostic test designed for rapid and simultaneous detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) from patients with positive blood cultures.** The test utilizes automated real-time polymerase chain reaction (PCR) to detect MRSA/SA DNA.
- The Xpert® MRSA/SA Blood Culture test is intended to aid in the detection and identification of MRSA/SA from positive blood culture bottles. The Xpert® MRSA/SA Blood Culture test 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 patient 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 test is not intended to monitor treatment for MRSA/SA infections.

Targets and Probes

Targets

- *Staphylococcus aureus* (SA)
- Methicillin-resistant *Staphylococcus aureus* (MRSA)

Probes

- probe for staphylococcal protein A (*spa*)
- the gene for methicillin resistance (*mecA*)
- staphylococcal cassette chromosome *mec* (SCC*mec*)

The targets are used singly or in combination to identify and differentiate SA and MRSA.

Xpert[®] MRSA/SA Blood Culture Requirements

GeneXpert[®] Systems

- GeneXpert[®] Dx Software **v 5.3** or higher
- Xpertise[™] Software **v 6.8** or higher

Test Kits

- GXMRSA/SABC-CE-10

Materials Required but not Provided

- Disposable transfer pipettes
- Vortex mixer
- Personal Protective Equipment (PPE)
- 1:10 Bleach
- 70% ethanol or denatured ethanol

Optional

- Uninterruptible Power Supply /Surge Protector
- Printer

Good Laboratory Practice

Personel 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 work surfaces are dry

Specimens, Samples, and Kits Storage

- Store specimens and sample away from kit to prevent contamination

Equipment(s)

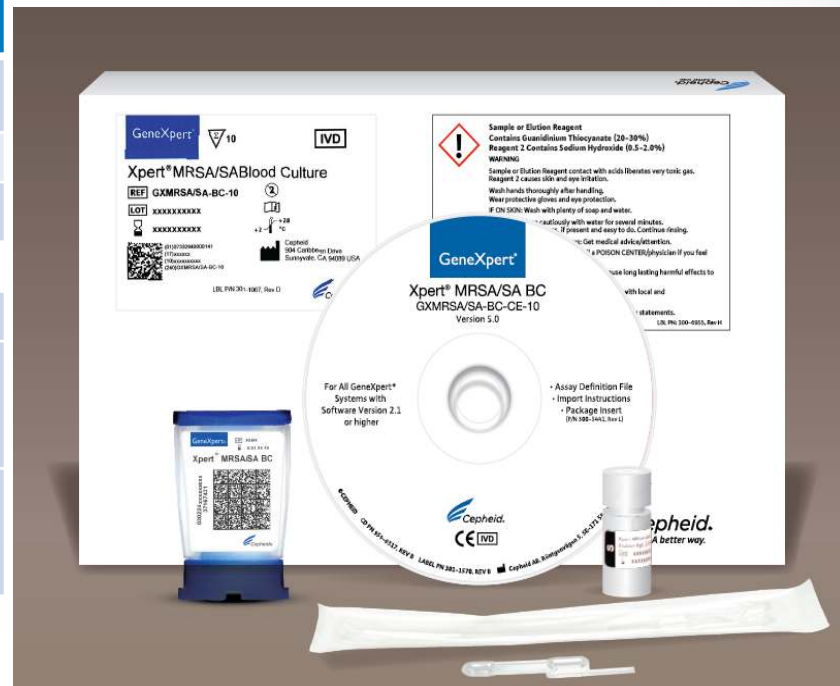
- Use filtered pipette tips when recommended
- Follow the manufacturer's requirements for calibration and maintenance of equipment(s)

Kit Handling



Xpert[®] MRSA/SA BC Kit Contents

Xpert [®] MRSA/SA BC Assay	
Catalog Number	GXMRSA/SABC-CE-10
Tests Per Kit	10
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



Cartridges contain chemically hazardous substances-please see Package Insert and Safety Data Sheet for more detailed information.

Xpert[®] MRSA/SA BC Kit 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
 - The following Blood culture media can be used in Xpert[®] MRSA/SA Blood Culture Assay:
 - BACTEC[™] PEDS PLUS[™]/F Medium
 - BACTEC[™] Plus Aerobic/F Medium
 - BACTEC[™] Plus Anaerobic/F Medium
 - BACTEC[™] Standard Anaerobic/F Medium
 - BACTEC[™] Standard/10 Aerobic/F Medium
 - BACTEC[™] LYTIC/10 Anaerobic/F Culture Vials
 - bioMérieux BacT/ALERT[®] SA standard aerobic
 - bioMérieux BacT/ALERT[®] SN standard anaerobic
 - VersaTREK[™] REDOX[™] 1R (aerobic)
 - VersaTREK[™] REDOX[™] 2R (anaerobic)
- 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



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

Warnings and Precautions

- 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.

Xpert Assay Limitations

- The performance of the Xpert[®] MRSA/SA Blood Culture Assay was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test. Results from the Xpert[®] MRSA/SA Blood Culture Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- Xpert[®] MRSA/SA Blood Culture Assay should be used only to test blood culture bottles that are positive for microbial growth and shown by Gram stain to contain Gram-positive cocci in clusters (GPCC) or single Gram-positive cocci (GPC).
- Blood culture media containing activated charcoal cannot be used with the Xpert[®] MRSA/SA Blood Culture Assay (e.g., BacT/ALERT FAN aerobic).

For detailed information, refer to the current Package Insert

Specimen Collection, Storage and Transport



Xpert[®] MRSA/SA Blood Culture Assay Collection

The following Blood culture media can be used in Xpert[®] MRSA/SA Blood Culture Assay:

BACTEC[™] PEDS PLUS[™]/F Medium
BACTEC[™] Plus Aerobic/F Medium
BACTEC[™] Plus Anaerobic/F Medium
BACTEC[™] Standard Anaerobic/F Medium
BACTEC[™] Standard/10 Aerobic/F Medium
BACTEC[™] LYTIC/10 Anaerobic/F Culture Vials
bioMérieux BacT/ALERT[®] SA standard aerobic
bioMérieux BacT/ALERT[®] SN standard anaerobic
VersaTREK[™] REDOX[™] 1R (aerobic)
VersaTREK[™] REDOX[™] 2R (anaerobic)

Specimen Collection, Transport and Storage

Specimen Aliquot	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	Up to 3 days
	Room Temperature	24 hours

- When positive for growth, remove blood culture bottles from incubation. A Gram stain must be performed from the positive blood culture following standard laboratory procedure.
- For positive blood culture bottles that show Gram-positive cocci in clusters (GPCC) or single Gram-positive cocci (GPC) by Gram stain, **collect approximately 1 mL of positive blood culture specimen** and label with Sample ID.
- Specimens which have been stored at room temperature for more than 24 hours or refrigerated at 2-8°C for more than three days should not be tested by Xpert[®] MRSA/SA Blood Culture Assay.

Cartridge Preparation



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.

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



- 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.



Run a Test

1 Create Test

GeneXpert



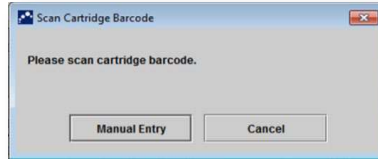
Start the test within **3 hours** after adding the sample to the cartridge

GeneXpert
Infinity



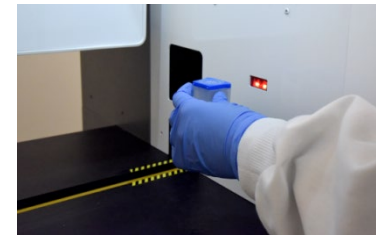
Place the cartridge on the conveyor within **30 minutes** of adding the sample.

2 Scan barcode : 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 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

7 Click on Start Test

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

Create Test

Patient ID
Sample ID
Patient ID 2
Last Name

Select Assay: Xpert MRSA/SA BC

Select Module: A3

Reagent Lot ID*: 16119 Expiration Date*: 2016/1/17

Test Type: Specimen

Sample Type: Other

Notes

Start Test Scan Cartridge Barcode



Create a Test on Xpertise™ Software

4 Complete the fields as required

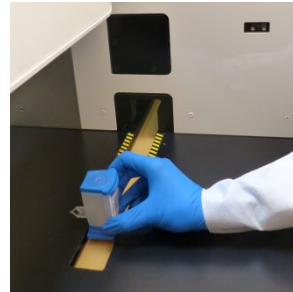
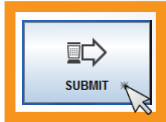
Order Test - Test Information

Patient ID patientid	
Sample ID sampleid	
Last Name patient	First Name id
Assay Xpert MRSA/SA BC	
Request Lot ID* 12102	Cartridge S/N* 282769448
Expiration Date* 2018/11/04	Priority Normal
Test Type Specimen	
Sample Type Other	Other Sample Type
Notes	

5 The Assay Name Protocol is selected automatically

6 Click on SUBMIT

7 Place the cartridge into the conveyor belt



Automated Xpert Protocol

1

Sample is added to the cartridge

2

The cartridge is loaded into the System

3

Nucleic acids are purified

Purified nucleic acids mix with the PCR reagents

4

Simultaneous amplification and detection occurs

5

Results are ready to view

6



Quality Controls



Assay Control Strategy

CONTROL

- **Xpert[®] MRSA/SA Blood Culture Quality Controls**
 - 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
 - Sample Processing Control (SPC)
 - Probe Check Controls (PCC)

Refer to 301-4868 *GeneXpert Quality Control Features for All Cepheid Xpert Assays*

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
 - reagent rehydration
 - probe integrity
 - PCR tube filling
 - dye stability

- **Sample Processing Controls (SPC)**

- non-infectious spore in each cartridge
 - Verifies adequate sample processing
 - Verifies lysis, presence of the organism and 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
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.

Result Interpretation



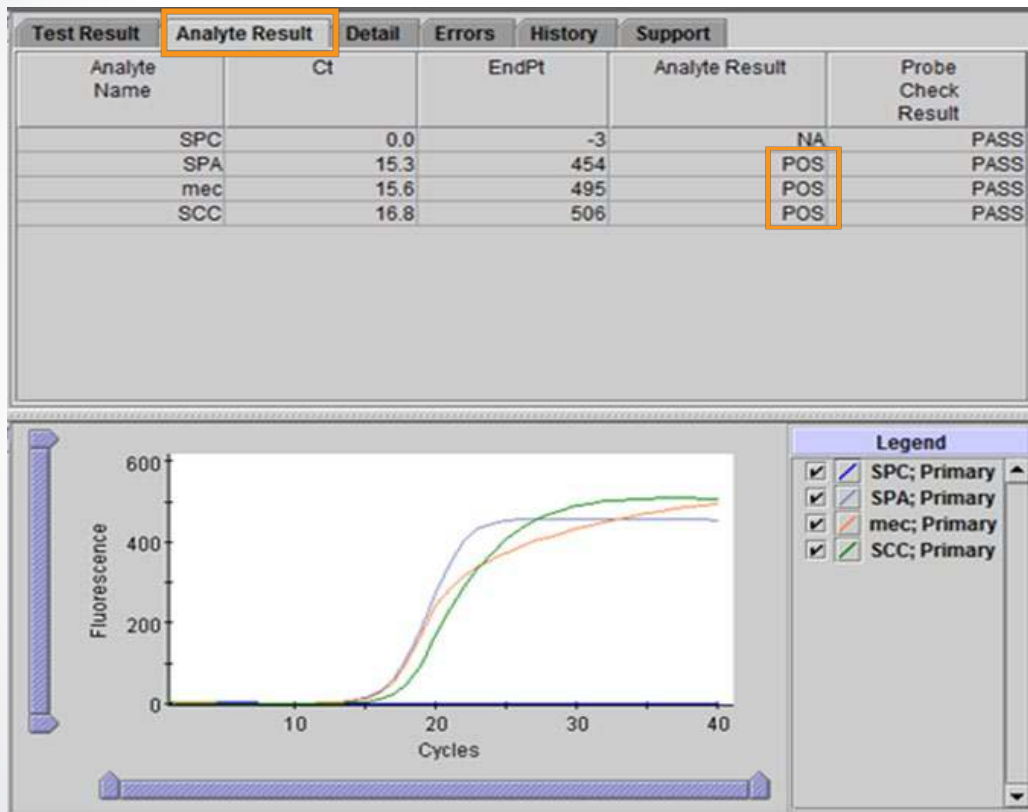
Results Summary

Result displayed	SPA	mecA	SCCmec	Other criteria	SPC
MRSA POSITIVE SA POSITIVE	+	+	+		+/-
	+	+	-	the rules-based algorithm conditions are met for the Ct values of <i>mecA</i> and <i>spa</i>	
	-	+	+	the rules-based algorithm conditions are met for the Ct values of <i>mecA</i> and <i>SCCmec</i>	
MRSA NEGATIVE	+	-	+/-		+/-
SA POSITIVE	-	+	+	the rules-based algorithm conditions are not met for the Ct values of <i>mecA</i> and <i>SCCmec</i>	
	+	+	-	the rules-based algorithm conditions are not met for the Ct values of <i>mecA</i> and <i>spa</i>	
MRSA NEGATIVE SA NEGATIVE	-	+/-	-		+/-
		+	+	the rules-based algorithm conditions are not met for the Ct values of <i>mecA</i> and <i>SCCmec</i>	+/-
		-	+/-		+/-
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



The MRSA target DNA sequences are detected/SA target DNA sequence is detected within the sample.

MRSA POSITIVE — any of the following conditions occur:

- all MRSA targets (*spa*, *mecA* and *SCCmec*) are present, or
- *SCCmec* is not present, the rules-based algorithm conditions are met for the Ct values of *mecA* and *spa*, or
- *spa* is not present, the rules-based algorithm conditions are met for the Ct values of *mecA* and *SCCmec*.

SPC — NA (not applicable); the SPC signal is not part of the results interpretation in this case because MRSA amplification may compete with this control.

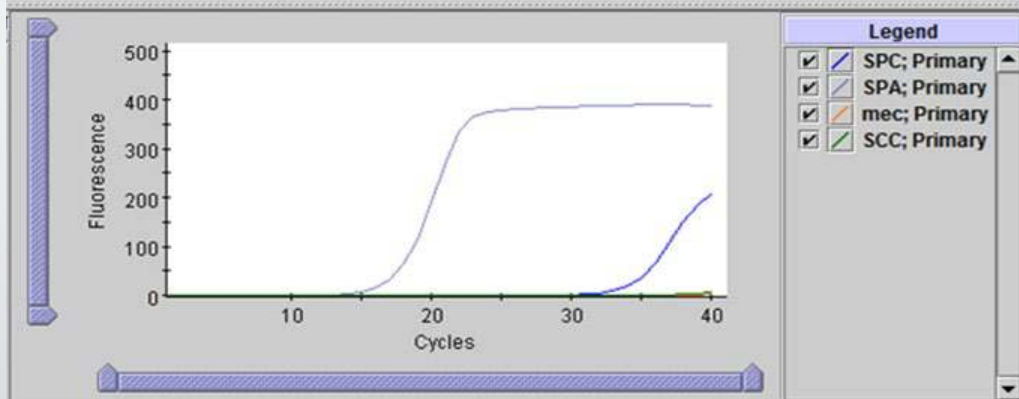
Probe Check — PASS; all probe check results pass

MRSA Negative / SA Positive

Test Result

MRSA NEGATIVE
SA POSITIVE

Test Result		Analyte Result	Detail	Errors	History	Support
Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result		
SPC	34.0	208	NA	PASS		
SPA	16.3	389	POS	PASS		
mec	0.0	3	NEG	PASS		
SCC	0.0	7	NEG	PASS		



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

SA POSITIVE — any of the following conditions occur:

- *spa* is present and *mecA* is not present,
or
- *spa* is not present, the rules-based algorithm conditions are not met for the Ct values of *mecA* and *SCCmec*,
or
- *SCCmec* is not present, the rules-based algorithm conditions are not met for the Ct values of *mecA* and *spa*.

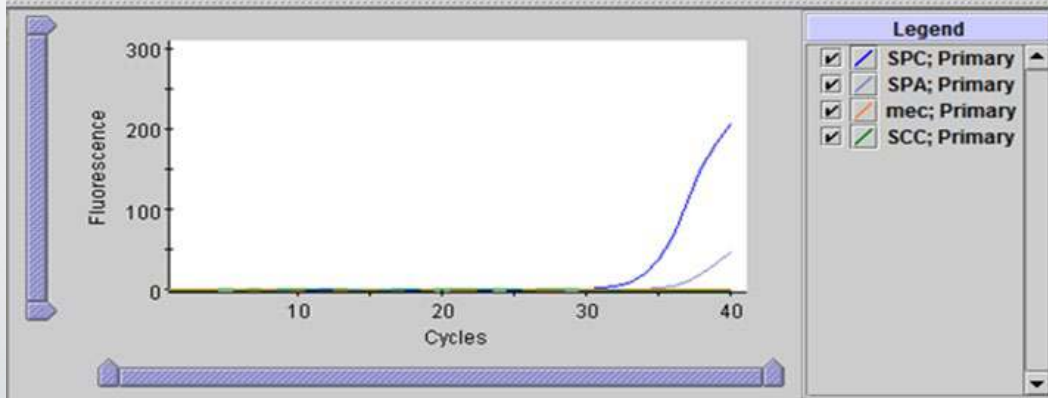
SPC — NA (not applicable); the SPC signal is not part of the results interpretation in this case because SA amplification may compete with this control.

Probe Check — PASS; all probe check results pass.

MRSA Negative / SA Negative

Test Result **MRSA NEGATIVE;
SA NEGATIVE**

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	34.0	206	PASS	PASS
SPA	38.1	47	NEG	PASS
mec	0.0	1	NEG	PASS
SCC	0.0	-2	NEG	PASS



SA target DNA sequence is not detected. SPC meets acceptance criteria.

NEGATIVE — the SA target (*spa*) is not present and any of the following conditions occur:

- *mecA* is not present,
or
- *SCCmec* is not present,
or
- Both *mecA* and *SCCmec* are present, the rules-based algorithm conditions are not met for the Ct values of *mecA* and *SCCmec*

SPC — PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting.

Or

SPC — N/A (not applicable); if any target analyte is positive, the SPC is ignored.

Probe Check — PASS; all probe check results pass

Troubleshooting

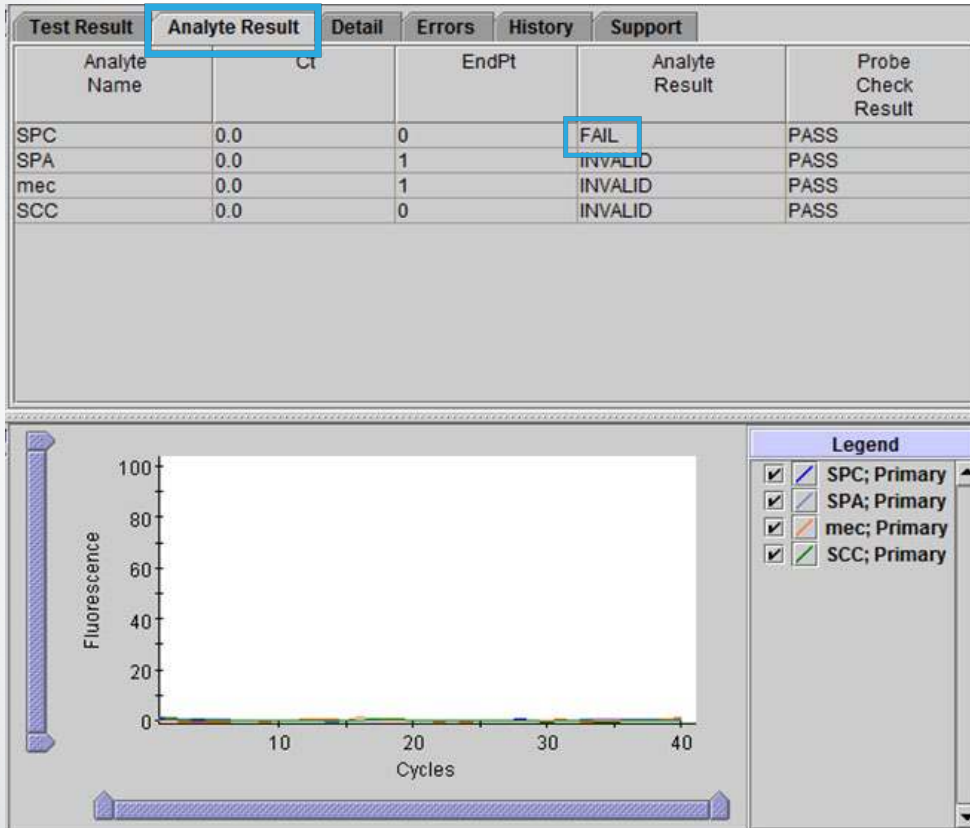


Factors That Negatively Affect Results

- Improper specimen collection
 - The bacterial load in the specimen is below the detection limit of the test
 - The performance of the Xpert[®] MRSA/SA Blood Culture Assay using blood culture bottle types other than those listed in the package insert have not been established.
- 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 Result

INVALID



Presence or absence of MRSA/SA target sequences cannot be determined, repeat test according to instructions in the section below. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR was inhibited.

INVALID — Presence or absence of SA DNA cannot be determined.

SPC-FAIL — SPC Ct is not within valid range and endpoint below minimum setting.

Probe Check — PASS; all probe check results pass.

Possible Causes

- Improper sample collection or preparation
- Presence of interfering substances in the sample

Solution

- Repeat the test with a new cartridge and new elution vial

ERROR Result

ERROR

Test Result	Analyte Result	Detail	Errors	History	Support
Troubleshoot					
#	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		

Presence or absence of MRSA/SA target sequences cannot be determined, repeat test according to instructions in the section below. An error could be due to an improperly filled reaction tube, a probe integrity problem, a system component error, or because the maximum pressure limits were exceeded.

MRSA — NO RESULT

SA — NO RESULT

SPC — NO RESULT

Probe Check — FAIL/PASS

*If the probe check passed, the error has been caused by a system component failure or the maximum pressure limit was exceeded.

Solution

- Repeat the test with a new cartridge and elution vial

NO RESULT

Test Result **NO RESULT**

Test Result	Analyte Result	Detail	Errors	History	Support
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	

The presence or absence of MRSA/SA cannot be determined.

- SPA, Mec, SCC: NO RESULT
- SPC: NO RESULT
- Probe Check: NA (not applicable)

Possible Causes

A NO RESULT indicates that insufficient data were collected.

- Test was stopped with stop test button
- Electrical failure

Solution

- Secure the power
- Repeat the test with a new cartridge

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.



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*



Thank You.



www.Cepheid.com

