GeneXpert
Powered By CEPHEID INNOVATION

Xpert® MRSA/SA Blood Culture Assay

REF **GXMRSA/SA-BC-CE-10**

Cepheid®

In Vitro Diagnostic Medical Device IVD CE

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Xpert® MRSA/SA Blood Culture Assay

In Vitro Diagnostic Use Only

1. Proprietary Name

Xpert® MRSA/SA Blood Culture Assay

2. Common or Usual Name

MRSA/SA Blood Culture Assay

3. Intended Use

The Cepheid Xpert MRSA/SA Assay performed in the GeneXpert® Dx System 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 Assay is intended to aid in the detection and identification of MRSA/SA from positive blood culture bottles. 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 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 Assay is not intended to monitor treatment for MRSA/SA infections.

4. Summary and Explanation

Staphylococcus aureus (SA) is a major nosocomial pathogen that causes a range of diseases including endocarditis, osteomyelitis, toxic shock syndrome, food poisoning, carbuncles and boils. In the early 1950s, acquisition and spread of beta-lactamase-producing plasmids thwarted the effectiveness of penicillin for treating S. *aureus* infections. In 1959, methicillin, a synthetic penicillin, was introduced. By 1960, methicillin-resistant S. *aureus* strains were identified. This was determined to be the result of S. *aureus* acquiring the *mecA* gene. In the US today, MRSA is responsible for approximately 25% of nosocomial infections and reports of community-acquired MRSA are increasing, resulting in significant morbidity and mortality. In an attempt to limit the spread of these infections, control strategies and policies are being developed and implemented in healthcare settings. Controlling MRSA is a primary focus of most hospital infection control programs. Currently, the standard surveillance method for detecting MRSA is culture, which is very laborious and time intensive.[1,2,3,4,5]

A rapid and more sensitive method for detection of MRSA and SA from positive Blood Culture Bottles will represent a definite advantage for patient management and the use of appropriate antibiotics for treatment.

5. Principle of the Procedure

The GeneXpert Dx System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR and RT-PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. For a full description of the system, see the *GeneXpert Dx System Operator Manual.*

The Xpert MRSA/SA Blood Culture Assay includes reagents for the detection of MRSA and SA as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert MRSA/SA Blood Culture Assay detect proprietary sequences for the staphylococcal protein A (*spa*), the gene for methicillin/oxacillin resistance (*mecA*), and the staphylococcal cassette chromosome *mec* (SCC*mec*) inserted into the SA chromosomal *attB* site.

6. Reagents and Instruments

6.1 Material Provided

The Xpert MRSA/SA Blood Culture Assay kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert MRSA/SA Blood Culture Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 per cartridge
• Reagent 1	3.0 ml per cartridge
• Reagent 2 (Sodium Hydroxide)	3.0 ml per cartridge
Xpert MRSA/SA BC Assay Elution Reagent pouch	**10 x 2.0 mL per pouch**
• Elution Reagent (Guanidinium thiocyanate)	
Disposable Small Transfer Pipettes	**12**
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
• Instructions for Use (Package Insert)	

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials

6.2 Storage and Handling

- Store the Xpert MRSA/SA Blood Culture Assay cartridges and reagents at 2 – 28°C.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge and reagents within 30 minutes after opening the cartridge lid.
- Do not use any reagents that have become cloudy or discolored.

7. Materials Required but Not Provided

- GeneXpert Dx System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.0 or higher, barcode wand reader, and Operator Manual
- Printer: If printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Disposable, sterile transfer pipettes

8. Materials Available but Not Provided

KWIK-STIKs™ from MicroBiologics catalog #0158MRSA and catalog #0360MSSA as positive controls and #0371MSSE (methicillin-sensitive *Staphylococcus epidermidis*) as negative control.

9. Warnings and Precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[6] and the Clinical and Laboratory Standards Institute.[7]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert MRSA/SA Blood Culture Assay does not provide susceptibility results. Additional time is required to culture and perform susceptibility testing.
- Do not substitute Xpert MRSA/SA Blood Culture Assay reagent with other reagents.
- Do not open the Xpert MRSA/SA Blood Culture Assay cartridge lid except when adding sample and reagent or performing a retest.
- Do not use a cartridge that has been dropped or shaken after you have added the sample and reagent.
- Do not use a cartridge that has a damaged reaction tube.

- Each single-use Xpert MRSA/SA Blood Culture Assay cartridge is used to process one test. Do not reuse spent cartridges.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring 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 procedure. 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.[11]
- 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 1® (aerobic)
    - VersaTREK REDOX 2® (anaerobic)
- Blood culture media containing activated charcoal cannot be used with the Xpert MRSA/SA Blood Culture Assay.
- Test only Blood Culture Bottles that are positive for microbial growth with the Xpert MRSA/SA Blood Culture Assay.

10. Chemical Hazards[9,10]

- UN GHS Hazard Pictogram: 
- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Causes skin irritation
  - Causes serious eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash thoroughly after handling.
    - Do not eat, drink, or smoke when using this product.
    - Avoid release to the environment.
    - Wear protective gloves/protective clothing/eye protection/face protection
  - **Response**
    - IF ON SKIN: Wash with plenty of soap and water.
    - Take off contaminated clothing and wash before reuse.
    - Specific treatment, see the supplemental first aid information.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persist: Get medical advice/attention
    - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician if you feel unwell.
    - Rinse mouth.
  - **Storage Disposal**
    - Dispose of content and/or container in accordance with local, regional, national, and/or international regulations

11. Specimen Collection, Transport and Storage

1. Upon positivity determination, remove blood culture bottles from incubation. A Gram stain should be performed from the positive blood culture following standard laboratory procedure. If you cannot remove the blood culture bottle from the instrument when it is first detected as positive, please remove it at your earliest convenience.
2. For positive blood culture bottles revealing Gram positive cocci in clusters (GPCC) or single gram positive cocci (GPC) by Gram Stain, remove a 1 mL aliquot of the well-mixed broth and label with Sample ID.

Note The results of blood cultures are critical to patient care. Please follow established guidelines and policies of your laboratory/institution for reporting positive blood culture results (verbal, written or electronic) to healthcare providers.

3. If not testing with the Xpert MRSA/SA Blood Culture Assay immediately, store the aliquot at 2 – 8 °C within 30 minutes of removal from the blood culture bottle. The positive blood culture aliquot should be tested by Xpert MRSA/SA Blood Culture Assay within 4 hours after removal from the positive bottle.

12. Microbiology Culture

For MRSA culturing methods, follow current laboratory standard operating procedures. For culturing, remaining untested swab specimens should be placed in appropriate transport systems and cultured within 4 days.

13. Procedure

13.1 Preparing the Cartridge

Important **Start the test within 15 minutes of adding the Elution reagent to the cartridge**.

To add the sample and Elution reagent into the cartridge:

1. Remove the cartridge and an Elution reagent vial from the kit.
2. Using the Small Transfer Pipette, transfer one drop of positive blood culture (50µL) into the Elution Reagent.

Note Use sterile gauze to handle swab to minimize risk of contamination.

3. Close the Elution vial lid and vortex at high speed for 10 seconds.
4. Open the cartridge lid. Using a sterile transfer pipette, transfer the entire contents of the Elution Reagent to the sample chamber of the Xpert MRSA/SA Blood Culture Assay cartridge.
5. Close the cartridge lid.

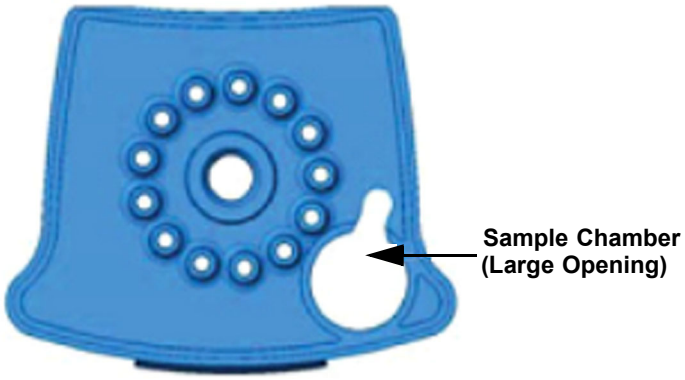

Figure 1. MRSA/SA Blood Culture Assay Cartridge (Top View)

13.2 Starting the Test

Important **Before you start the test, make sure the Xpert MRSA/SA Blood Culture Assay definition file is imported into the software**.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual.*

Note The steps you follow can be different if the system administrator changed the default workflow of the system.

1. Turn on the GeneXpert Dx instrument and then turn on the computer. The GeneXpert software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Dx System software using your user name and password.
3. In the GeneXpert Dx System window, click Create Test. The Create Test window opens.
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window.
6. Scan the barcode on the Xpert MRSA/SA Blood Culture Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert MRSA /SA Blood Culture Assay cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test.** In the dialog box that appears, type your password.
8. Open the instrument module door with the blinking green light and load the cartridge.
9. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
10. Wait until the system releases the door lock before opening the module door and removing the cartridge.
11. Dispose of the used cartridges in an appropriate specimen waste container according to your institution's standard practices.

14. Viewing and Printing Results

This section list the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual*.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

15. Quality Control

15.1 Built-in Quality Controls

CONTROL

Each test includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

- **Sample processing control (SPC)** — Ensures the sample was correctly processed. The SPC contains spores of *Bacillus globigii* in the form of a dry spore cake that is included in each cartridge to verify adequate processing of Xpert MRSA/SA Blood Culture Assay sample. The SPC verifies that lysis of Staphylococcus aureus has occurred if the organisms are present and verifies that specimen processing is adequate. Additionally this control detects specimen-associated inhibition of the real-time PCR assay. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Probe check control (PCC)** — Before the start of the PCR reaction, the GeneXpert Dx System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

15.2 External Controls

KWIK-STIKs™ (MicroBioLogics, catalog #0158MRSA and catalog #0360SA as positive controls and #0371MSSE as negative control) may be used for training, proficiency testing and external QC of the GeneXpert Dx System. External controls may be used in accordance with local, state, federal accrediting organizations, as applicable. Follow the MicroBioLogics external control procedure described below:

1. Tear open the pouch at notch and remove the KWIK-STIK.
2. Pinch the bottom of the ampoule in the cap to release the hydrating fluid.
3. Hold vertically and tap to facilitate flow of fluid through shaft into bottom of unit containing pellet.
4. To facilitate dissolution of the lyophilized cell pellet, crush the pellet and gently pinch the bottom chamber.
5. Pull apart the KWIK-STIK to release the swab, and insert the swab into the tube containing the Elution Reagent (black cap).
6. The KWIK-STIK swab is now ready for Xpert MRSA/SA Blood Culture Assay testing.

16. Interpretation of Results

The results are interpolated by the GeneXpert Dx System from measured fluorescent signals and embedded calculation algorithms and will be shown in the **View Results** window. Possible results are:

Table 1. Xpert MRSA/SA Blood Culture Results and Interpretation

Result	Interpretation
MRSA POSITIVE/SA POSITIVE Figure 2	MRSA target DNA sequences are detected/SA target DNA sequence is detected. • MRSA POSITIVE — all MRSA targets have a Ct within the valid range and endpoint above the minimum setting. • SPC — NA (not applicable); SPC is ignored because MRSA amplification may compete with this control. • Probe Check — PASS; all probe check results pass.

Table 1. Xpert MRSA/SA Blood Culture Results and Interpretation (Continued)

Result	Interpretation
MRSA NEGATIVE/SA POSITIVE	• MRSA target DNA sequences are not detected/SA target DNA sequence is detected. • SA POSITIVE — the SA target has a Ct within the valid range and endpoint above the minimum setting. • SPC — NA (not applicable); SPC is ignored because SA amplification may 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 Figure 3	• *Staphylococcus aureus* target DNA sequence is not detected. SPC meets acceptance criteria. • NEGATIVE —*Staphylococcus aureus* target DNA is not detected. • SPC — PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting. • Probe Check — PASS; all probe check results pass.
INVALID Figure 4	• Presence or absence of MRSA/SA target sequences cannot be determined, repeat test with new sample. SPC does not meet acceptance criteria, the sample was not properly 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 within valid range and endpoint below minimum setting. • Probe Check — PASS; all probe check results pass.
ERROR	• Presence or absence of MRSA/SA cannot be determined, repeat test with new sample. The Probe Check control failed which is probably due to an improperly filled reaction tube a probe integrity problem, or because the maximum pressure limits were exceeded. • MRSA — NO RESULT • SA — NO RESULT • SPC — NO RESULT • Probe Check — FAIL*; one or more 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/SA target DNA sequences cannot be determined, repeat test according to instructions in the section below. Insufficient data were collected to produce a test result. For example, this can occur if the operator stopped a test that was in progress. • MRSA — NO RESULT • SA — NO RESULT • SPC — NO RESULT • Probe Check — NA (not applicable)

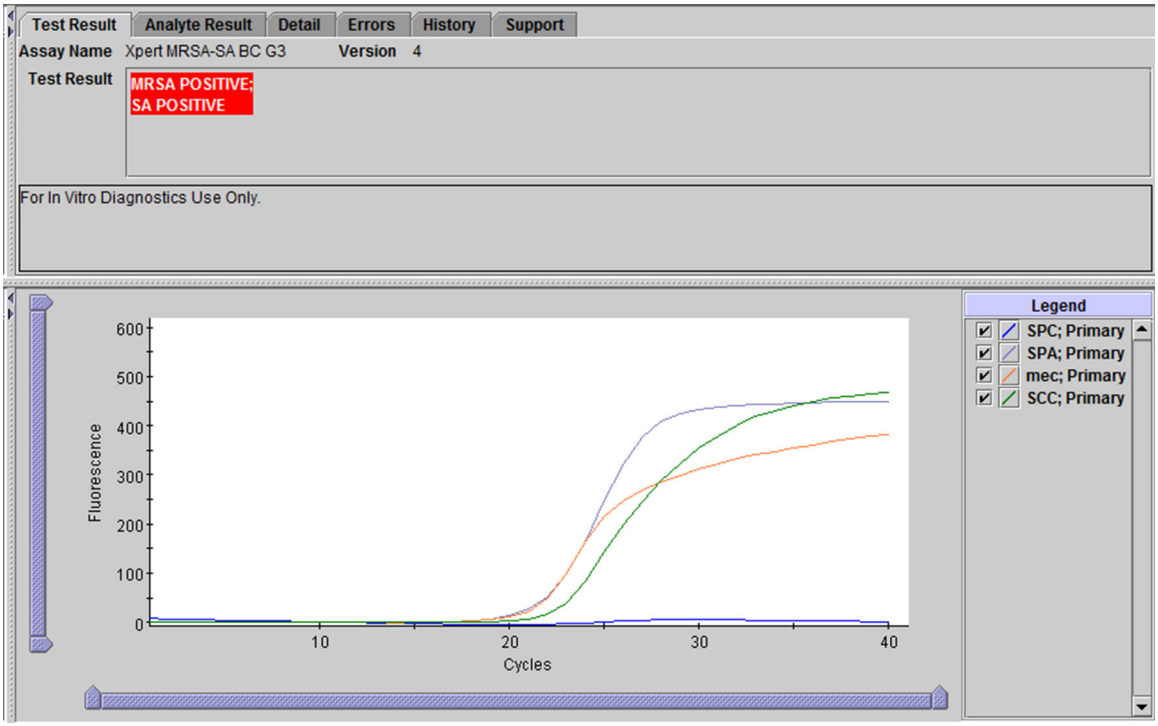

Figure 2. An Example of an MRSA Positive Result

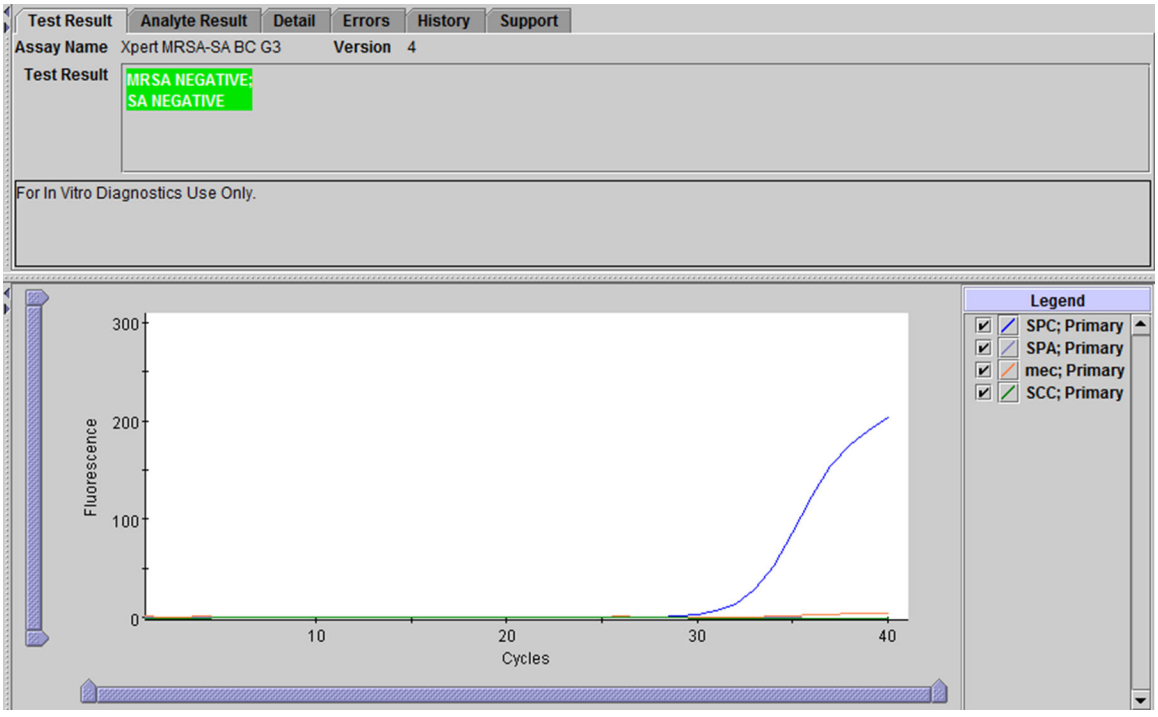

Figure 3. An Example of a Negative Result

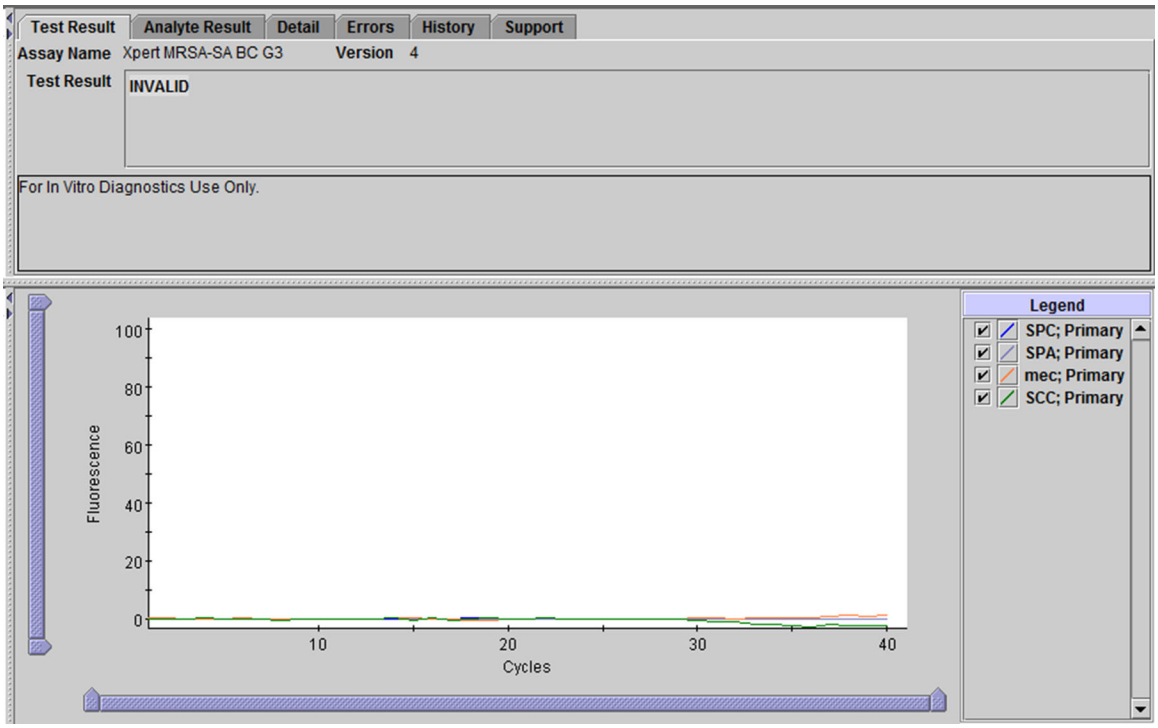

Figure 4. An Example of an Invalid Result

17. Reasons to Repeat the Assay

17.1 Reason to Repeat the Test

If any of the test results mentioned below occur, repeat the test using a new cartridge (do not re-use the cartridge). Perform the retest procedure within 3 hours of an indeterminate result.

- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed or PCR is inhibited.
- An **ERROR** result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the 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. To perform a retest:
  - Transfer remaining contents from the sample chamber to a new Elution Reagent.
  - Vortex and add the entire contents of the Elution Reagent to the sample chamber of the new MRSA/SA Blood Culture Assay cartridge.
  - Close the lid and start new test.

17.2 Retest Procedure

If an External QC fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

To perform a retest:

If retesting within 3 hours of an indeterminate result*:

1. Transfer remaining contents from the sample chamber to a new elution reagent using a disposable transfer pipette.
2. Vortex and add the entire contents of the elution reagent to the sample chamber of the new MRSA/SA Blood CultureAssay cartridge.
3. Close the lid and start new test.

*If the retest cannot be performed within 3 hours, use a new sample.

18. 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.
- Blood culture media containing activated charcoal cannot be used with the Xpert MRSA/SA Blood Culture Assay.
- The Xpert MRSA/SA Blood Culture Assay may generate false negative MRSA results when testing borderline oxacillin resistant *S. aureus* (BORSA). The mechanism of oxacillin resistance in BORSA strains is due to an increased production of B-lactamases, not the *mecA* gene. BORSA with oxacillin MICs of 4-8 µg/mL are considered borderline resistant but, would be reported as MRSA negative by the Xpert MRSA/SA Blood Culture Assay. BORSA strains are rare in the United States.
- The Xpert MRSA/SA Blood Culture Assay may generate false negative MRSA results when testing modified *S. aureus* (MOD-SA). The mechanism of oxacillin resistance in MOD-SA strains is due to changes in affinity of penicillin binding proteins for oxacillin, not the *mecA* gene. MOD-SA with oxacillin MICs of 4-8 µg/mL are considered borderline resistant but, would be reported as MRSA negative by the Xpert MRSA/SA Blood Culture Assay. MOD-SA strains are rare in the United States. Erroneous test results might occur from improper specimen collection, failure to follow the recommended sample collection, handling and storage procedures, technical error, sample mix-up, or because the number of organisms in the specimen is too low to be detected by the test. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- Because the detection of MRSA and SA is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MRSA or SA.
- Testing with the Xpert MRSA/SA Blood Culture Assay should be used as an adjunct to other methods available.
- Mutations or polymorphism in primer or probe binding regions may affect detection of new or unknown MRSA variants resulting in a false negative result.
- In a mixed culture containing both MRSA and SA, the LoD of MRSA is variable when extremely high concentrations of SA are present. Competition from SA was observed at a MRSA:SA ratio of $1:1x10^6$.
- The Xpert MRSA/SA Blood Culture test may generate a false positive MRSA result when testing a specimen containing both methicillin-resistant coagulase negative staphylococci (MRCNS) and methicillin-susceptible Staphylococcus aureus.
- As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the LoD of the assay may be detected, but results may not be reproducible.
- Xpert MRSA/SA Blood Culture Assay results may sometimes be **INVALID** due to a failed SPC control, **ERROR** or **NO RESULT**, and require retesting that can lead to a delay in obtaining final results.
- Because of the dilution factor associated with the retest procedure, it is possible that MRSA or SA positive specimens, very near or at the limit of detection (LoD) of the Xpert MRSA/SA Blood Culture Assay, may result in a false negative result upon retest.

19. Interfering Substances

A study was performed to assess potentially inhibitory effects, if any, of substance(s) encountered in positive blood cultures using the Xpert MRSA/SA Blood Culture Assay. Potentially inhibitory substances may include, but are not limited to blood and components of blood culture media. Substances were tested undiluted in replicates of three with MRSA cells spiked near the analytical Limit of Detection (~2.5 x LoD) and higher (~10 x LoD).

- No inhibitory effects were observed in the presence of BACTEC™ (Becton Dickinson) standard aerobic/anaerobic soybean-casein digest broth containing the anticoagulant SPS or their "PLUS" aerobic/anaerobic media containing ion exchange and nonionic adsorbent resins to remove antimicrobials when compared to buffer controls.
- No inhibitory effects were observed in the presence of BacT/ALERT® (bioMerieux) standard aerobic/anaerobic tryptic soy broth containing the anticoagulant SPS when compared to buffer controls.
- No inhibitory effects were observed in the presence of whole blood when compared to buffer controls.

20. Performance Characteristics

20.1 Clinical Performance

Performance characteristics of the Xpert MRSA/SA Blood Culture Assay were determined in a multi-site prospective investigation study at five institutions (4 United States and 1 European Union) by comparing the MRSA/SA Blood Culture Assay on the GeneXpert System (Xpert MRSA/SA Assay) with culture. Subjects included individuals whose blood cultures were positive for growth. The study included samples from nine different types of adult blood culture bottles and one pediatric bottle. Blood culture bottles containing charcoal were excluded.

An aliquot from each blood culture bottle was tested by the Xpert MRSA/SA Blood Culture Assay and by culture. Culture methods varied among centers, though oxacillin/methicillin susceptibility was determined at all centers by disk diffusion test using a 30 µg cefoxitin disk and cutoff of 21/22 mm.

Assay performance of the Xpert MRSA/SA Blood Culture Assay was calculated relative to the culture results.

20.2 Overall Results

A total of 406 specimens were tested for MRSA and SA by Xpert and culture; 212 US and 194 EU.

The Xpert MRSA/SA Blood Culture Assay identified 98.3% of the specimens positive for MRSA and 99.4% of the specimens negative for MRSA relative to the culture method. For the specimens tested, the MRSA positive predictive value was 96.6% and the MRSA negative predictive value was 99.7%.

The Xpert MRSA/SA Blood Culture Assay identified 100% of the specimens positive for SA and 98.6% of the specimens negative for SA relative to the culture method. For the specimens tested, the SA positive predictive value was 96.7% and the SA negative predictive value was 100%.

Table 2. MRSA — US and EU Centers Combined

		Culture +	Culture -			
Xpert MRSA/SA Blood Culture Assay	+	57	2	59	Sens	98.3%
	-	1[a]	346	347	Spec	99.4%
		58	348	406		

[a] The one false negative specimen attained in the Xpert MRSA/SA Blood Culture Assay testing was further investigated by the PBP2a latex agglutination test (Oxiod, UK) using standard laboratory methods. Results of the aforementioned test showed that this isolate overproduced penicillinase and was misidentified by culture as MRSA.

Table 3. SA — US and EU Centers Combined

		Culture +	Culture -			
Xpert MRSA/SA Blood Culture Assay	+	120	4	124	Sens	100%
	-	0	282	282	Spec	98.6%
		120	286	406		

21. Analytical Performance

21.1 Analytical Specificity

Cultures from 98 American Type Culture Collection (ATCC) and 7 Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) strains representing species phylogenetically related to *Staphylococcus aureus* or those potentially encountered in a hospital environment, 29 strains of methicillin-sensitive coagulase negative staphylococci, and 9 strains of methicillin-resistant coagulase negative staphylococci were tested using the Xpert MRSA/SA Blood Culture Assay. The organisms tested were represented by 74 Gram positive, 28 Gram negative, 3 yeast, 95 aerobic and 10 anaerobic species. Two or more replicates of each isolate were tested at 1.7-3.2 McFarland units. Under the conditions of the study, all isolates were reported MRSA negative and SA negative, none of the isolates were detected by the Xpert MRSA/SA Blood Culture Assay. Positive and Negative controls were included in the study. The specificity was 100%.

21.2 Analytical Ubiquity (Inclusivity)

The analytical ubiquity (inclusivity) of the Xpert MRSA/SA Blood Culture Assay was determined using 25 *Staphylococcus aureus* strains supplied by Dr. Fred C. Tenover at the Centers for Disease Control and Prevention (CDC). These specimens are reported to be representative of MRSA and MSSA strains currently encountered in the healthcare community. All strains were tested in triplicate using 100 µL stationary phase cell suspensions diluted 10 million-fold. The panel consists of MRSA strains representing SCC*mec* types II, IV, IVa, IVb, and IVc in addition to several unknown types. Data supplied by the CDC indicate these strains, when characterized by pulsed-field gel electrophoresis (PFGE), represent numerous USA types including USA 100, the most common hospital-acquired strain and USA 300 and 400, the most common community-acquired strains.[8]

As shown in Table 4, all MRSA strains were correctly reported MRSA positive and SA positive using the Xpert MRSA/SA Blood Culture Assay. Additionally, each MSSA strain was correctly reported MRSA negative and SA positive. After CHROMagar and Xpert MRSA/SA Blood Culture Assay results were reported to the CDC, they revealed that the Xpert MRSA/SA Blood Culture Assay did not incorrectly identify specimen 95:99. Specimen 95:99 was mislabeled by the CDC. Specimen 95:99 was correctly reported MRSA negative and SA negative by the Xpert MRSA/SA Blood Culture Assay. Colony forming units per assay were determined by plate counts in duplicate.

Table 4. Analytical Ubiquity of the Xpert MRSA/SA Blood Culture Assay

Lab ID	Sender	Source	PFGE Type	SCC*mec* Type	CHROMagar MRSA Result	Xpert MRSA/SA BC Assay Result	SPC Ct	*spa* Ct	*mecA* Ct	SCC Ct	CFU per Assay
94:1013	VT	Skin lesion	USA1000	IV	+	MRSA POSITIVE; SA POSITIVE	34.7	30.7	31	32.6	152
95:99[a]	CT	Blood	USA500	IV	-	MRSA NEGATIVE; SA NEGATIVE	34.1	0	0	0	37
96:308	NM	Stool	USA900	MSSA	-	MRSA NEGATIVE; SA POSITIVE	34	29.4	0	0	201
96:281	NC	Blood	USA200	II	+	MRSA POSITIVE; SA POSITIVE	33.4	33.6	34	35.3	101
148-99	NY	Blood	USA600	II	+	MRSA POSITIVE; SA POSITIVE	34.3	33.2	33.1	35.2	43
182-99	MN	Unknown	USA400	IVa	+	MRSA POSITIVE; SA POSITIVE	43.7	26.7	27.1	28.7	417
18626	OH	Blood	USA100	II	+	MRSA POSITIVE; SA POSITIVE	34.8	30.7	31	32.7	138

Table 4. Analytical Ubiquity of the Xpert MRSA/SA Blood Culture Assay (Continued)

Lab ID	Sender	Source	PFGE Type	SCC*mec* Type	CHROMagar MRSA Result	Xpert MRSA/SA BC Assay Result	SPC Ct	*spa* Ct	*mecA* Ct	SCC Ct	CFU per Assay
0:50	TN	Stool	USA600	not typed	+	MRSA POSITIVE; SA POSITIVE	33.6	31.2	31.4	33.2	115
0-25-4	MS	Nasal	USA700	IVa	+	MRSA POSITIVE; SA POSITIVE	35.5	29.1	29.3	30.9	178
0-25-37	MS	Skin/Soft Tissue	USA300	IVa	+	MRSA POSITIVE; SA POSITIVE	34.7	32.3	32.7	34.2	94
1-1-81	WA	Nasal	USA400	not typed	+	MRSA POSITIVE; SA POSITIVE	34.3	33	33.7	35.5	106
1-1-493	WA	Wound	USA800	IV	+	MRSA POSITIVE; SA POSITIVE	33.7	31.5	31.7	33.4	113
N7129	NHANES	Nasal	USA900	MSSA	-	MRSA NEGATIVE; SA POSITIVE	34.3	29.9	0	0	84
107-03	NV	Blood	USA200	not typed	+	MRSA POSITIVE; SA POSITIVE	34	33	33.3	34.9	99
GA201	GA-ABC	Unknown	USA100	II	+	MRSA POSITIVE; SA POSITIVE	33.6	32.3	32.4	34	95
GA217	GA-ABC	Unknown	USA300	IVb	+	MRSA POSITIVE; SA POSITIVE	33.6	30.8	31.2	33	121
GA229	GA-ABC	Unknown	USA500	IV	+	MRSA POSITIVE; SA POSITIVE	37.8	31.7	31.9	33.3	81
7031	AK	Abscess	USA1100	IVa	+	MRSA POSITIVE; SA POSITIVE	34.2	30.8	31.5	32.9	73
102-04	CA	Nasal	USA1200	MSSA	-	MRSA NEGATIVE; SA POSITIVE	33.9	29.4	0	0	110
8-03	WI	Unknown	USA700	not typed	+	MRSA POSITIVE; SA POSITIVE	33.3	29	29.2	30.9	202
510-04	Uruguay	Abscess	USA1100	IVc	+	MRSA POSITIVE; SA POSITIVE	34.6	31.5	32	33.8	143

Table 4. Analytical Ubiquity of the Xpert MRSA/SA Blood Culture Assay (Continued)

Lab ID	Sender	Source	PFGE Type	SCC*mec* Type	CHROMagar MRSA Result	Xpert MRSA/ SA BC Assay Result	SPC Ct	*spa* Ct	*mecA* Ct	SCC Ct	CFU per Assay
27-05	HI	Wound	USA800	IVc	+	MRSA POSITIVE; SA POSITIVE	40.7	27.8	28.1	29.8	373
CA46	CA	Blood	USA1000	IV	+	MRSA POSITIVE; SA POSITIVE	33.4	32.6	33.7	35.8	81
398-05	HI	Wound	USA1000	IVb	+	MRSA POSITIVE; SA POSITIVE	33.6	32.8	33.4	35.9	59
N4151	NHANES	Nasal	USA800	IVb	+	MRSA POSITIVE; SA POSITIVE	34	30.7	31.2	32.9	101

[a] Specimen 95:99: After CHROMagar and Xpert MRSA/SA Blood Culture Assay results were reported to the CDC, they revealed that the Xpert MRSA/SA Blood Culture Assay correctly identified specimen 95:99. Specimen 95:99 was mislabeled by the CDC. Specimen 95:99 was correctly reported MRSA negative and SA negative by the Xpert MRSA/SA Blood Culture Assay. Ct values represent the mean of three replicates. The information contained in the grey columns was provided to Cepheid by Dr. Fred C. Tenover from the CDC.

21.3 Analytical Sensitivity

Additional studies were performed to determine the 95% confidence interval for the analytical limit of detection (LoD) of this assay. The limit of detection is defined as the lowest number of colony forming units (CFU) per sample that can be reproducibly distinguished from negative samples with 95% confidence. A maximum valid cycle of 36.0 is set for both MRSA and SA data analysis. Any spa, mecA, or SCC result with a Ct value greater than 36.0 is reported negative. For MRSA (type II cells), replicates of 20 were evaluated at seven concentrations (0, 50, 75, 100, 125, 150 and 200 CFU/sample). For SA, replicates of 20 were evaluated at six concentrations (0, 20, 25, 40, 50 and 60 CFU/sample).

Under the conditions of the study and using a maximum valid Ct setting of 36.0, results indicate that the LoD point estimate for SA is 48.0 CFU/sample with a 95% confidence interval ranging from 42.4 CFU to 57.2 CFU. The estimate and confidence levels were determined using logistic regression with data (number of positives per number of tests at each level) taken at six levels (0, 20, 25, 40, 50 and 60 CFU/sample). Note that the analytical LoD for SA will be conservatively reported as 58 CFU/ 50 µL sample.

The LoD point estimate for MRSA is 109.4 CFU/sample with a 95% confidence interval ranging from 98.8 CFU to 128.2 CFU. The estimate and confidence levels were determined using logistic regression with data (number of positives per number of tests at each level) taken at seven levels (0, 50, 75, 100, 125, 150 and 200 CFU/sample). Note that the analytical LoD for MRSA will be conservatively reported as 130 CFU/ 50µL sample.

The confidence intervals were determined using maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix.

22. References

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23. Cepheid Headquarters Locations

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24. Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

25. Table of Symbols

Symbol	Meaning
	Catalog number
	*In vitro* diagnostic medical device
	Do not reuse
	Batch code
	CE marking – European Conformity
	Consult instructions for use
	Caution
	Manufacturer
	Country of Manufacture
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
CH REP	Authorized Representative in Switzerland
	Importer

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna
Sweden

Product of Sweden

CH REP

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
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CE IVD