GeneXpert
Powered By CEPHEID INNOVATION

Xpert® MRSA/SA Blood Culture

REF **GXMRSA/SABC-CE-10**

Instructions for Use

IVD CE

Cepheid

In Vitro Diagnostic Medical Device

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Trademark, Patents and Copyright Statements

See Section 23, Revision History, for a description of changes.

Xpert® MRSA/SA Blood Culture

For *In Vitro* Diagnostic Use

1 Proprietary Name

Xpert® MRSA/SA Blood Culture

2 Common or Usual Name

Xpert MRSA/SA Blood Culture

3 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.

4 Summary and Explanation

Staphylococcus aureus (SA) is a human pathogen, which is the causative agent of a range of diseases including bacteremia, 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 SA infections. In 1959, methicillin, a semi-synthetic penicillin, was introduced and, soon after, methicillin-resistant SA (MRSA) strains were identified. Resistance is now known to be conferred when SA acquires the *mec*gene complex, which contains the *mecA* gene and potentially other *mecA* variants such as $mecA_{LGA251}$ referred to as *mecC*. In the United States today, MRSA is responsible for approximately 25% of healthcare associated infections, resulting in significant morbidity and mortality.

Significant attributable mortalities have been reported for MRSA and methicillin-susceptible SA (MSSA) bacteremias. Currently, the standard method for detecting SA including MRSA from blood culture bottles is by *in vitro* culture. Public health may benefit from a rapid and sensitive method of testing for SA, including MRSA.[1,2,3,4,5,6]

5 Principle of the Procedure

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid purification and amplification, and detection of the target sequence in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require 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 minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert MRSA/SA Blood Culture test 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 indicate the presence of inhibitor(s) in the PCR reaction. The SPC also ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. An additional internal control, 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 test detect proprietary sequences for the staphylococcal protein A (*spa*), the gene for methicillin resistance (*mecA*), and the staphylococcal cassette chromosome mec (SCC*mec*), which is inserted into the SA chromosome at the *attB* site. The targets are used singly or in combination to identify and differentiate SA and MRSA.

For MRSA present in a blood culture bottle in the absence of any other bacterial species, the assay utilizes rules-based algorithms where the cycle threshold (Ct) values of the three targets (*spa*, *mecA*, and SCC*mec*) are compared to designate whether the targets are derived from the same MRSA organism. MRSA is considered present when: 1) all three targets have Ct values within the valid range and endpoints above the minimum setting, 2) in the absence of SCC*mec*, the rules-based algorithm conditions are met for the Ct values of *mecA* and *spa*, or 3) in the absence of *spa*, the rules-based algorithm conditions are met for the Ct values of *mecA* and SCC*mec*.

6 Reagents and Instruments

6.1 Material Provided

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

Xpert MRSA/SA Blood Culture Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried) • Reagent 1 • Reagent 2 (Sodium Hydroxide)	1 of each per cartridge 3 mL per cartridge 3 mL per cartridge **10 x 2.0 mL**
Xpert MRSA/SA Blood Culture Elution Reagent (Guanidinium Hydrochloride and surfactants)	**12**
Disposable Fixed Volume (50pL) Transfer Pipettes **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 cartridges and reagents at 2 – 28°C.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open the cartridge lid until you are ready to perform testing.

6.3 Materials Required but Not Provided

- GeneXpert Dx System or GeneXpert Infinity System (catalog number varies by configuration): GeneXpert instrument, computer, barcode wand reader and Operator Manual
  - For GeneXpert Dx System: GeneXpert Dx software version 5.3 or higher
  - For GeneXpert Infinity-80 and Infinity-48s Systems: Xpertise software version 6.8 or higher
- Printer: If printer is required, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Vortex mixer
- Disposable transfer pipettes (for sample transfer to cartridge)

6.4 Materials Available but Not Provided

KWIK-STIKs™ from MicrobioLogics catalog # 0158MRSA (SCC*mec* type II) and catalog # 0360MSSA (*Staphylococcus aureus* subsp. *aureus*) may be used as external positive controls, and catalog # 0371MSSE (methicillin-susceptible *Staphylococcus epidermidis*) as external negative control.

7 Warnings and Precautions

- For *in vitro* diagnostic use.
- 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[8] and the Clinical and Laboratory Standards Institute.[8]
- 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 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. Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents.[9]
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- The Xpert MRSA/SA Blood Culture test does not provide antimicrobial susceptibility testing results. Additional subculturing of all positive blood cultures should be performed for susceptibility testing.
- 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.
- Do not substitute Xpert MRSA/SA Blood Culture elution reagent with other reagents.
- Do not use a cartridge that has been dropped or shaken.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use any reagents that have become cloudy or discolored.
- Do not use a new cartridge that has leaked. Liquid on the outside of a spent cartridge may indicate a problem.
- Each single-use Xpert MRSA/SA Blood Culture test cartridge is used to process one test. Do not reuse spent cartridges.
- The following Blood culture media can be used in Xpert MRSA/SA Blood Culture test:
  - 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)

- Blood culture media containing activated charcoal cannot be used with the Xpert MRSA/SA Blood Culture test.
- Xpert MRSA/SA Blood Culture test 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).

8 Specimen Collection, Transport and Storage

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.

- 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.
- If the specimen will be tested within 24 hours, refrigerate at 2-8°C or store at room temperature. If the specimen will be tested after 24 hours, refrigerate at 2-8°C for up to three days. 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 test.

9 Chemical Hazards[10, 11]

- 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.
    - 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 persists: 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.

10 Procedure

10.1 Preparing the Cartridge

Important **If using a GeneXpert Dx System, start the test within 3 hours of adding the prepared sample to the cartridge. If using a GeneXpert Infinity System, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the sample to the cartridge. Remaining shelf-life is tracked by the system by the Xpertise Software so that tests are initiated prior to the three hour on-board expiration.**

To add the sample to the cartridge:

1. Remove the cartridge and elution reagent from the package.
2. Gently mix the blood culture sample by hand. Do not vortex.
3. Using the supplied fixed volume pipette (50µL), transfer the contents of the fixed volume pipette containing the positive blood culture sample to the elution reagent vial by following the steps below:
   a. Firmly squeeze the top bulb of the pipette.
   b. While still squeezing, place the pipette tip into the sample.
   c. With the pipette still in the sample, release pressure on the bulb to fill pipette.
   d. Place pipette tip over mouth of the elution reagent vial.
   e. Firmly squeeze the top bulb to empty the contents of the pipette into the Elution Reagent vial. It is normal for excess liquid to remain in the overflow bulb.

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

4. Close the elution reagent cap and vortex at high speed for 10 seconds.
5. Open the cartridge lid. Using a transfer pipette (not supplied), transfer the entire contents of the elution reagent to the sample chamber of the Xpert MRSA/SA Blood Culture test cartridge. See Figure 1.
6. Close the cartridge lid and start the test.

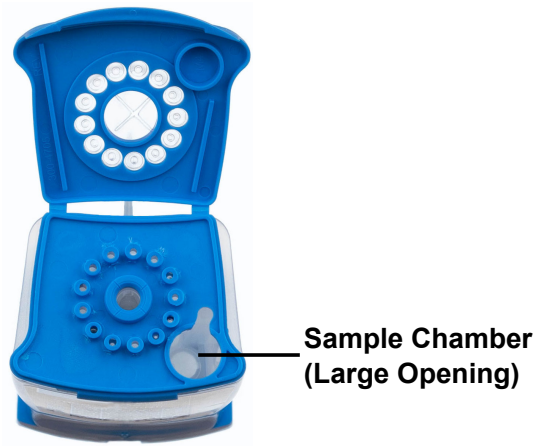

Figure 1. MRSA/SA Blood Culture Cartridge (Top View)

10.2 Running the Test

- For the GeneXpert Dx System, see Section 10.2.1.
- For the GeneXpert Infinity System, see Section 10.2.2.

10.2.1 GeneXpert Dx System
Starting the Test

Before you start the test, make sure that:

Important
- The system is running the correct GeneXpert Dx software version shown in section - Materials Required but Not Provided.
- The correct assay definition file is imported into the software.

This section lists the basic steps for running the test. 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 System, then turn on the computer and log on. The GeneXpert software will launch automatically. If it does not, double-click the GeneXpert Dx software shortcut icon on the Windows® desktop.
2. Log on using your username and password.
3. In the **GeneXpert System** window, click **Create Test**.
   The **Create Test** window displays. The **Scan Patient ID barcode** dialog box displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly.
   The Patient ID is associated with the test results and displays in the **View Results** window and all the reports. The **Scan Sample ID barcode** dialog box displays.
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 displays in the **View Results** window and all the reports. The **Scan Cartridge Barcode** dialog box displays.
6. Scan the barcode on the 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 cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

7. Click **Start Test**. In the dialog box that displays, type your password, if required.
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, then remove the cartridge.
11. Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

Viewing and Printing Results

This section lists 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.

10.2.2 GeneXpert Infinity System
Starting the Test

Before you start the test, make sure that:

Important
- The system is running the correct Xpertise software version shown in section - Materials Required but Not Provided.
- The correct assay definition file is imported into the software.

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Infinity System Operator Manual*.

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

1. Power up the instrument. The Xpertise software will launch automatically. If it does not, double-click the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the computer, then log on to the GeneXpert Xpertise software using your user name and password.
3. In the **Xpertise Software Home** workspace, click **Orders** and in the **Orders** workspace, click **Order Test**. The **Order Test - Patient ID** workspace displays.
4. Scan or type in the Patient ID. If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and displays in the **View Results** window and all the reports.
5. Enter any additional information required by your institution, and click the **CONTINUE** button. The **Order Test - Sample ID** workspace displays.
6. 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 displays in the **View Results** window and all the reports.
7. Click the **CONTINUE** button. The **Order Test - Assay** workspace displays.
8. Scan the barcode on the 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 cartridge does not scan, then repeat the test with a new cartridge. If you have scanned the cartridge barcode in the software and the assay definition file is not available, a screen displays indicating the assay definition file is not loaded on the system. If this screen displays, contact Cepheid Technical Support.

After the cartridge is scanned, the **Order Test - Test Information** workspace displays.

9. Verify that the information is correct, and click **Submit**. In the dialog box that displays, type your password, if required.
10. Place the cartridge on the conveyor belt. The cartridge automatically loads, the test runs, and the used cartridge are placed into the waste container.

Viewing and Printing Results

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

1. In the **Xpertise Software Home** workspace, click the **RESULTS** icon. The Results menu displays.
2. In the Results menu, select the **VIEW RESULTS** button. The **View Results** workspace displays showing the test results.
3. Click the **REPORT** button to view and/or generate a PDF report file.

11 Quality Control

11.1 Built-in Quality Controls

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

- **Sample Processing Control (SPC)** — The SPC is intended to indicate whether the sample was processed within specified operating conditions. The SPC contains spores of Bacillus globigii in the form of a dry spore bead that is included in each cartridge to verify adequate processing of Xpert MRSA/SA Blood Culture sample. The SPC verifies that lysis of SA 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 reactions and acts as an internal positive control. The SPC signal 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. The test will be Invalid if the SPC is not detected in a negative sample.
- **Probe Check Control (PCC)** — Before the start of the PCR reaction, the GeneXpert Instrument Systems measure the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if the data meets the assigned acceptance criteria.

11.2 External Controls

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

KWIK-STIKs (Microbiologics, catalog # 0158 MRSA [SCC*mec* type II] and catalog # 0360 MSSA as positive controls and # 0371 MSSE as negative control) may be used for training and external QC of the GeneXpert Instrument System. Follow the Microbiologics external control procedure described below:

1. Tear open the pouch at notch and remove the KWIK-STIK.
2. To release the hydrating fluid, pinch the bottom of the ampoule at the top of the KWIK-STIK cap until you hear the ampoule break.
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 mix in fluid using a pinching action. Feel the sides of the KWIK-STIK to confirm that the pellet is no longer palpable.
5. Pull apart the KWIK-STIK to release the swab, and break the swab into the tube containing the elution reagent (screw cap).
6. Close the elution lid and vortex at high speed for 10 seconds.
7. Continue with subsequent testing steps starting at Step 5 of Section 10.1, Preparing the Cartridge.
8. If the External QC fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

12 Interpretation of Results

The GeneXpert Instrument Systems generate the results from measured fluorescent signals and calculation algorithms used by the GeneXpert Instrument Systems software. The results can be seen in the **View Results** window. See Table 1 and Figure 2, Figure 3, Figure 4, and Figure 5.

For MRSA present in a blood culture bottle in the absence of any other bacterial species, the assay utilizes rules-based algorithms where the cycle threshold (Ct) values of the three targets (*spa*, *mecA*, and *SCCmec*) are compared to designate whether the targets are derived from the same MRSA organism.

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

Result	Interpretation

Result	Interpretation
MRSA POSITIVE/SA POSITIVE (Figure 2)	MRSA POSITIVE/SA POSITIVE — If any of the following conditions occur: ● all MRSA targets (*spa, mecA* and SCC*mec*) are present, or ● SCC*mec* 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 SCC*mec*. ● 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 (Figure 3)	MRSA NEGATIVE/SA POSITIVE – If 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 ● SCC*mec* 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 (Figure 4)	● MRSA NEGATIVE/SA NEGATIVE — The SA target (spa) is not present and if any of the following conditions occur: ● *mecA* is not present, or ● SCC*mec* 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 SCC*mec*. ● SPC — PASS; SPC has a Ct within the valid range and endpoint above the endpoint minimum setting. Or, if any target analyte is positive, the SPC is ignored. ● Probe Check — PASS; all probe check results pass.
INVALID (Figure 5)	● 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 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 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*; one or more of the probe check results fail. ● * If the probe check passed, the error has been caused by a system component failure or the maximum pressure limit was exceeded.

Result	Interpretation
NO RESULT	• Presence or absence of MRSA/SA target 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)

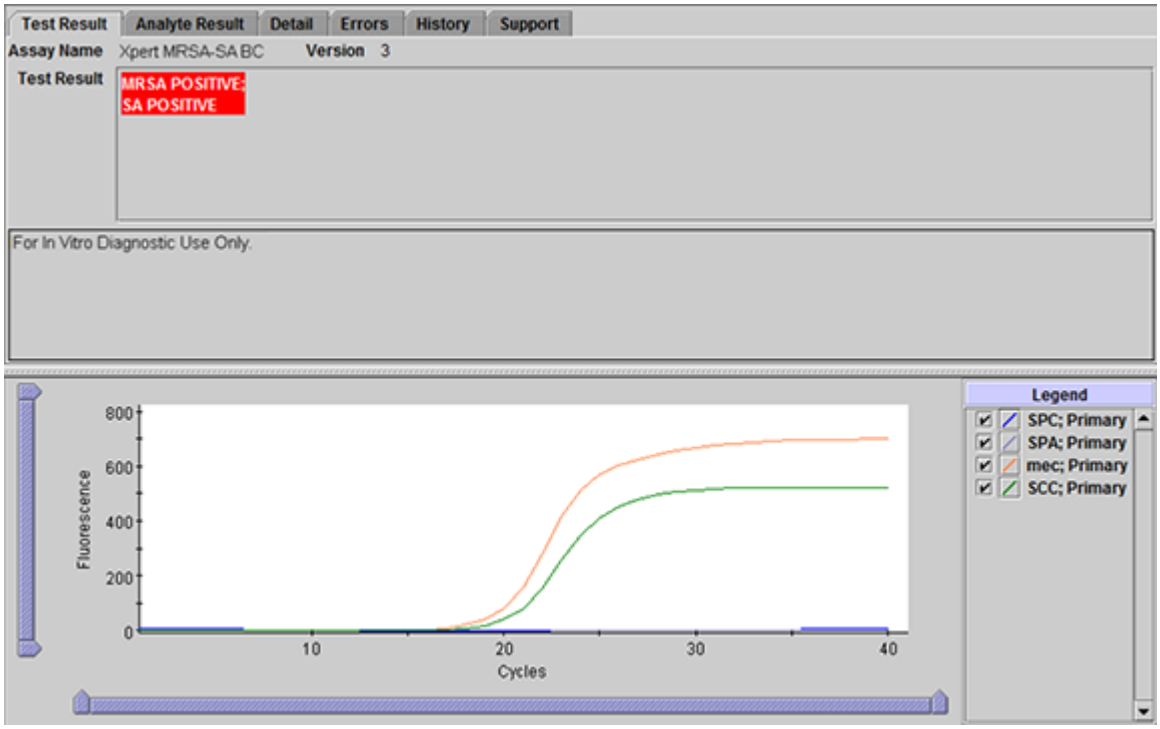

Figure 2. An Example of an MRSA Positive Result

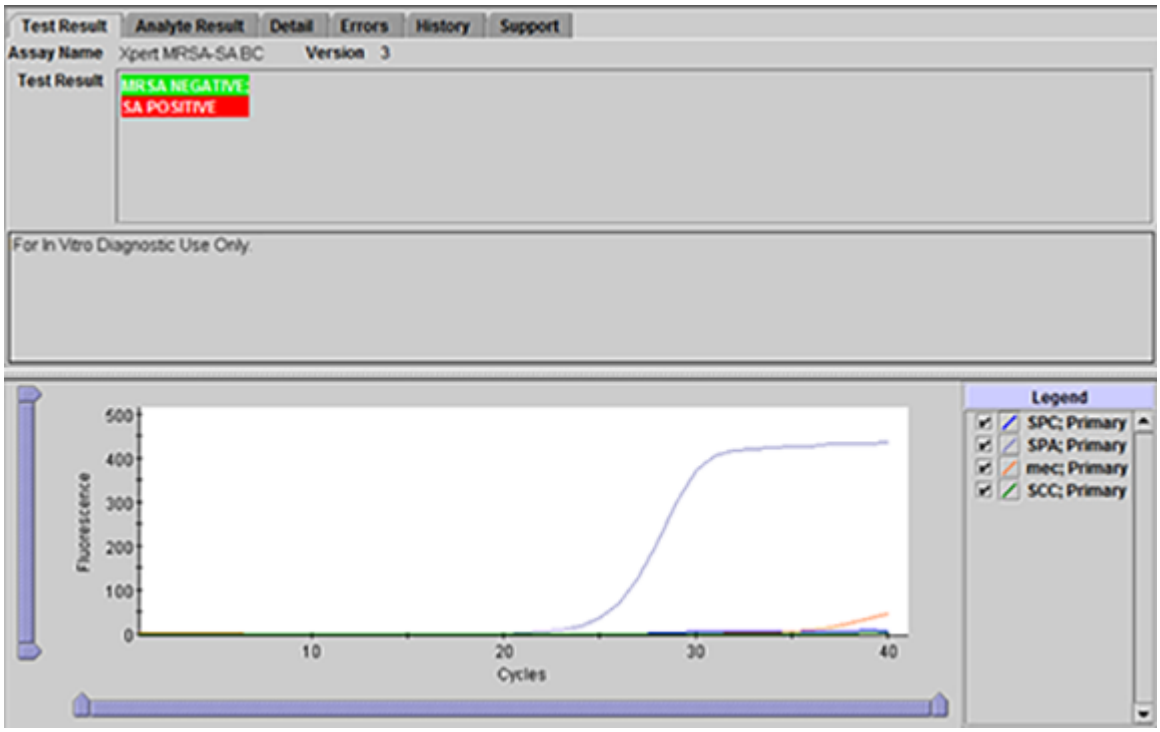

Figure 3. An Example of a SA Positive Result

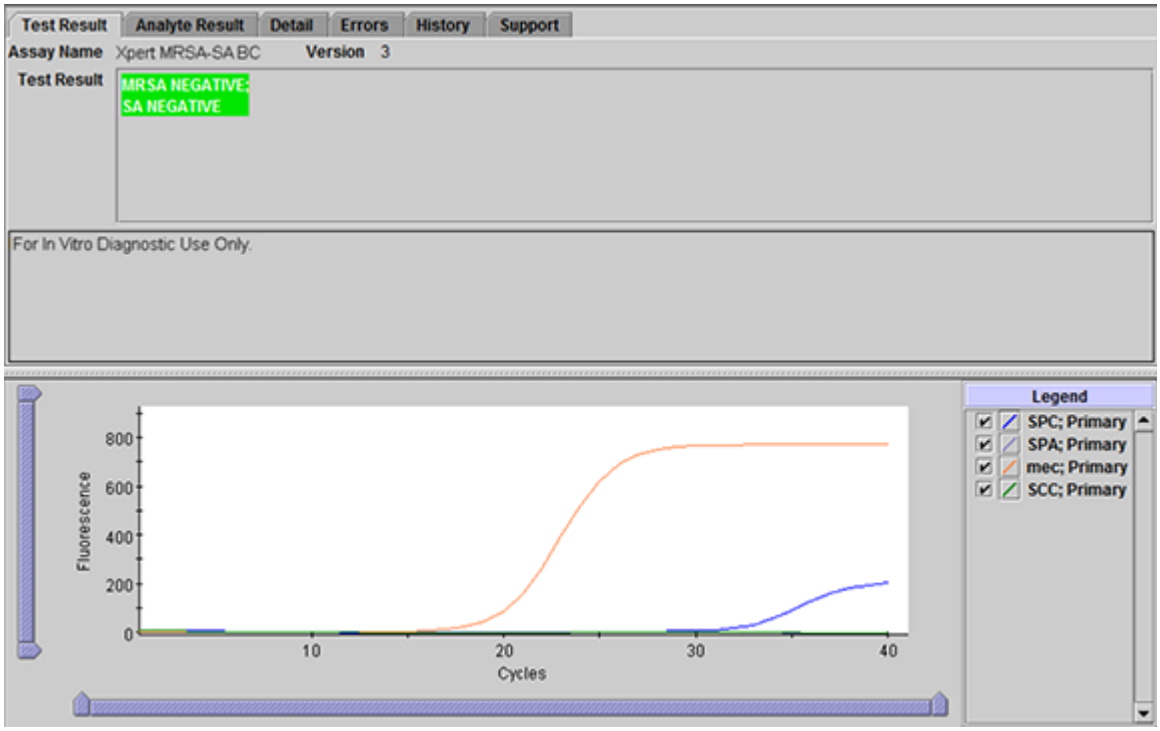

Figure 4. An Example of a Negative Result

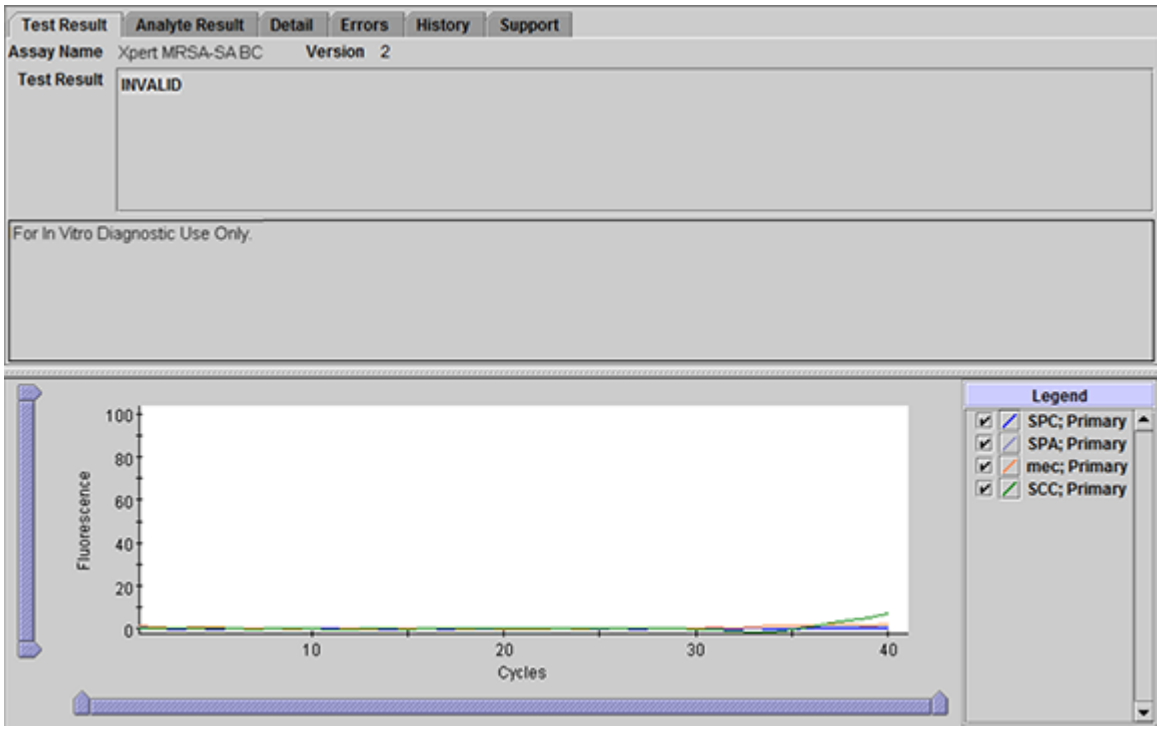

Figure 5. An Example of an Invalid Result

12.1 Reasons to Repeat the Test

The specimen should be retested if any of the following results are obtained from the first test.

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

12.2 Retest Procedure

Repeat the test using a new cartridge (do not re-use the cartridge) and new elution reagent vial.

When using a GeneXpert Dx instrument, start the test within 3 hours of adding the prepared sample to the cartridge. When using a GeneXpert Infinity system, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the sample to the cartridge. Remaining shelf-life is tracked by the system by the Xpertise Software so that tests are initiated prior to the three hour on-board expiration.

1. Remove the cartridge and elution reagent from the package.
2. Gently mix the blood culture sample by hand. Do not vortex.
3. Using the supplied fixed volume pipette (50µL), transfer the contents of the fixed volume pipette containing the positive blood culture sample to the Elution Reagent vial by following the steps below:
   a. Firmly squeeze the top bulb of the pipette.
   b. While still squeezing, place the pipette tip into the sample.
   c. With the pipette still in the sample, release the pressure on bulb to fill pipette.
   d. Place the pipette tip over mouth of elution reagent vial.
   e. Firmly squeeze the top bulb to empty the contents of the pipette into the Elution Reagent vial. It is normal for excess liquid to remain in the overflow bulb.

4. Close the elution reagent cap and vortex at high speed for 10 seconds.
5. Open the cartridge lid. Using a transfer pipette (not supplied), transfer the entire contents of the Elution Reagent vial to the Sample chamber of the Xpert MRSA/SA Blood Culture cartridge. See Figure 1.
6. Close the cartridge lid and start the test.

13 Limitations

- The performance of the Xpert MRSA/SA Blood Culture test 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 test should be interpreted in conjunction with other laboratory and clinical data available to the clinician.
- The performance of the Xpert MRSA/SA Blood Culture test using blood culture bottle types other than the BD BACTEC PEDS PLUS/F, BACTEC Plus Aerobic/F, BD BACTEC Plus Anaerobic/F, BD BACTEC Standard Anaerobic/F, BD BACTEC Standard/ 10 Aerobic/F, BD BACTEC LYTIC/10 Anaerobic/F, BacT/ALERT SA (Standard Aerobic), BacT/ALERT SN (Standard anaerobic), VersaTREK REDOX 1 (Aerobic), and VersaTREK REDOX 2 (Anaerobic) blood culture bottles has not been established.
- Blood culture media containing activated charcoal cannot be used with the Xpert MRSA/SA Blood Culture test (e.g., BacT/ALERT FAN aerobic).
- Testing with the Xpert MRSA/SA Blood Culture test should be used as an adjunct to other methods available.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MRSA variants resulting in a false negative result.
- 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.
- Xpert MRSA/SA Blood Culture test results may sometimes be INVALID, ERROR, or NO RESULT, and require retesting that can lead to a delay in obtaining final results.
- Target concentrations below the LoD of the assay may be detected, but results may not be reproducible.
- The Xpert MRSA/SA Blood Culture test may generate a false negative MRSA result when testing borderline oxacillin resistant SA(BORSA). The mechanism of oxacillin resistance in BORSA strains may be due to other factors (e.g., increased production of β- lactamase) rather than the presence of the mecA gene. BORSA with oxacillin MICs of 4-8 µg/mL are considered borderline resistant but may be reported as MRSA negative by the Xpert MRSA/SA Blood Culture test.
- The Xpert MRSA/SA Blood Culture test may generate a false negative MRSA result when testing modified SA (MOD-SA). The mechanism of oxacillin resistance in MOD-SA strains is due to other factors (e.g., changes in affinity of penicillin binding proteins for Oxacillin) rather than presence of 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 test.
- The Xpert MRSA/SA Blood Culture test will generate a false negative MRSA result when testing a strain containing a *mecA* homologue known as *mecC*, such as SA LGA251.
- 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*.
- The Xpert MRSA/SA Blood Culture test may generate a false negative MRSA result when testing a blood culture specimen containing multiple strains.
- A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of MRSA or SA.

14 Expected Values

In the Xpert MRSA/SA Blood Culture clinical study, a total of 792 blood culture specimens from eight sites across the United States were tested. The number and percentage of positive specimens as determined by the reference culture method is calculated by age group and presented in Table 2.

Table 2. Observed Prevalence of MRSA and SA by Culture

Age Group	Total N	MRSA by Culture		SA by Culture	
		Number Positive	Observed Prevalence	Number Positive	Observed Prevalence
0-20 years	22	2	9.1%	7	31.8%
21-30 years	43	8	18.6%	10	23.3%
31-40 years	65	8	16.9%	25	38.5%
41-50 years	124	22	17.7%	45	36.3%
51-60 years	154	23	14.9%	48	31.8%
61-70 years	165	15	19.1%	46	27.9%
>70 years	219	24	11.0%	54	24.7%
Total	792	105	13.3%	236	29.8%

15 Performance Characteristics

The updated Assay Definition File with rules-based algorithms and release of new GeneXpert software to support this update have been validated by the re-analyses of the original clinical performance data and a subset of the original analytical performance data, including LoD, inclusivity, exclusivity, potential interfering substances, reproducibility, and precision. The re-analyses showed the devices were substantially equivalent.

15.1 Clinical Performance

Performance characteristics of the Xpert MRSA/SA Blood Culture test were established in a multi-site prospective study at eight US institutions by comparing the Xpert MRSA/SA Blood Culture test with culture.

Subjects included individuals whose routine care called for blood culture testing. If the blood culture sample was positive for microbial growth and the Gram stain showed Gram-positive cocci (singles or in clusters), the sample was eligible for inclusion in the clinical study, and aliquots of leftover culture material were obtained for testing by the Xpert MRSA/SA Blood Culture test. Culture and Gram stain procedures, and patient management continued at the sites per the standard practice.

Susceptibility testing was performed in accordance with the CLSI documents M2-A11 and M100-S22.[12,13] Cefoxitin disc diffusion results were used as a surrogate for detecting methicillin/oxacillin resistance.

Performance of the Xpert MRSA/SA Blood Culture test was calculated as percent agreement with the reference culture results.

15.2 Overall Results

A total of 792 specimens were tested for MRSA and SA by Xpert MRSA/SA Blood Culture test and culture.

When compared to the reference culture method, the Xpert MRSA/SA Blood Culture test identified 98.1% of the specimens positive for MRSA and 99.6% of the specimens negative for MRSA.

When compared to the reference culture method, the Xpert MRSA/SA Blood Culture test identified 99.6% of the specimens positive for SA and 99.5% of the specimens negative for SA.

The performance of the Xpert MRSA/SA Blood Culture test is summarized in Table 3.

Table 3. Xpert MRSA/SA BC Performance vs. Reference Culture

		Culture			
		MRSA+	**SA+/MRSA-**	**Neg/No Growth**	**Total**
Xpert	MRSA +	103	2	1	106
	SA+/MRSA-	2	128	2	132
	SA-	0	1	553	554
	Total	105	131	556	792
Xpert Performance	MRSA PPA: 98.1% (103104/105, 95% CI: 93.3-99.8) NPA: 99.6% (684/687, 95% CI: 98.7-99.9)				
	SA PPA[a]: 99.6% (235/236, 95% CI 97.7-99.9) NPA[b]: 99.5% (553/556, 95% CI[c]: 98.4-99.9)				

[a] Positive Percent Agreement
[b] Negative Percent Agreement
[c] Confidence Interval

Of the Xpert MRSA/SA Blood Culture test runs on eligible specimens, 96.1% (764/795) were successful on the first attempt. The remaining 31 runs gave indeterminate results on the first attempt . 1: INVALID, 22: ERROR and 8: NO RESULT). Thirty of the 31 indeterminate cases were retested; one specimen was not retested. Twenty-eight of the 30 indeterminate cases that were retested yielded valid results upon repeat assay. The overall rate of assay success was 99.6% (792/795).

16 Analytical Performance

16.1 Limit of Detection

Studies were performed to determine point estimates and the two-sided 95% confidence intervals for the analytical limit of detection (LoD) of SA cells and methicillin-resistant SA (MRSA) cells diluted into a simulated negative blood culture matrix that can be detected by the Xpert MRSA/SA Blood Culture test. The matrix consisted of SA-free whole blood and MSSE (methicillin-susceptible Staphylococcus epidermidis) cells at 10^6 CFU/mL added to blood culture medium. 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 or the lowest concentration at which 19 of 20 replicates were positive.

For MRSA, 20 replicates were evaluated at each MRSA concentration tested (CFU/test) for 10 individual isolates representing SCC*mec* types I, II, III, IVa, IVd, V, VI, VII, and VIII. When characterized by pulsed-field gel electrophoresis (PFGE), USA100, the most common healthcare-acquired strain and USA400, one of the most common community-acquired strains, were represented.

For SA, 20 replicates were evaluated at each SA concentration (CFU/test) for 3 individual SA isolates. USA types USA900 and USA1200 were represented.

Point estimates and confidence intervals were determined by probit regression using data (i.e., the number of positive results per number of replicates at each level) spanning a range of CFU/test loadings. The confidence intervals were determined using maximum likelihood estimates on the probit model parameters using the large sample variance-covariance matrix. The LoD point estimates and 95% upper and lower confidence intervals for each SA and each MRSA SCC*mec* type tested are summarized in Table 4 and Table 5.

Table 4. LoD and 95% Confidence Intervals - SA

SA Strain	PFGE ID	Confirmed LoD (CFU/test) [at least 19/20 positive]	LoD Estimate (Probit Regression Analysis) (CFU/test)		
			Lower 95% CI	LoD Estimate	Upper 95% CI
102-04[a]	USA1200	100 (19/20)	60.4	74.5	101.6
29213[b]	unknown	150 (19/20)	120.1	138.2	172.7
N129[a]	USA900	300 (19/20)	224.2	255.2	314.8

[a] Strain Source: American Type Culture Collection (ATCC), Manassas, VA USA

[b] Strain Source: Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

Table 5. LoD and 95% Confidence Intervals - MRSA

MRSA Strain ID	PFGE ID	Confirmed LoD (CFU/test) [at least 19/20 positive]	LoD Estimate (Probit Regression Analysis) (CFU/test)		
			Lower 95% CI	LoD Estimate	Upper 95% CI
Type I (64/4176)[a]	USA500	350 (19/20)	332.3	366.8	433.5
Type II (N315)[b]	USA100[c]	175 (19/20)	113.7	137.0	178.1
Type III (11373)[b]	unknown	225 (19/20)	191.9	222.6	273.9
Type IVa (MW2)[b]	USA400[c]	350 (19/20)	313.1	356.1	427.0
Type V (ST59)[d]	USA1000[c]	250 (19/20)	218.2	243.1	282.3
Type VI (HDE288)[ef]	USA800[c]	250 (19/20)	222.2	246.0	385.0
Type VII (JCSC6082)[a]	unknown	300 (19/20)	264.1	288.0	347.1
Type VIII (WA MRSA-16)[d]	unknown	400 (19/20)	348.7	386.7	499.1
Type II (BK2464)[b]	USA100[g]	125 (19/20)	94.3	116.1	162.0
Type IVd (BK2529)[bf]	USA500[g]	200 (19/20)	120.8	148.8	202.5

[a] Teruyo Ito, Department of Bacteriology, School of Medicine Juntendo University, Tokyo, Japan
[b] Barry Kreiswirth, Director Public Health Research Institute (PHRI), Newark, NJ, USA
[c] K. Bonnstetter et al., J Clin Micro 2007, p. 141-146; L. McDougal et al., J Clin Micro 2003, p. 5113-5120
[d] Geoffrey Coombs, Department of Microbiology and Infectious Diseases, Royal Perth Hospital, Perth, WA
[e] Hermina deLencastre, Laboratory of Molecular Genetics, Instituto de Tecnologia Quimica e Biologica (ITQB), Universidade Nova de Lisboa, Oeiras, Portugal
[f] Heterogeneous oxacillin-resistant isolates
[g] Barry Kreiswirth, personal communication

The results of this study indicate that the Xpert MRSA/SA Blood Culture test will produce a positive SA result 95% of the time in a positive blood culture aliquot (50 µL) containing 300 CFU and a positive MRSA result 95% of the time for a positive blood culture aliquot (50 µL) containing 400 CFU.

16.2 Analytical Inclusivity Study (Reactivity)

Two hundred fifty (250) SA strains (47 MSSA and 203 MRSA) from multiple sources were tested using the Xpert MRSA/SA Blood Culture test. Selections were made to represent the primary lineages with emphasis placed on the specific clonal complexes within which MRSA is predominantly observed. Lineages that contain MRSA and MSSA, as well as those that contain MSSA exclusively were included. When characterized by pulsed-field gel electrophoresis (PFGE), numerous USA types including USA100, the most common healthcare-acquired strain, and USA300 and USA400, the most common community- acquired strains, were also included.[14] Strains representing "Empty Cassette" variants and heterogeneous strains identified as borderline oxacillin-resistant SA (e.g., Oxacillin MIC values of 4-8 µg/mL) or BORSA were also tested.

All strains were tested in triplicate using 10 µl of stationary phase cell suspension diluted 1 million-fold. Colony forming units per assay (CFU/test) were determined by plate counts in triplicate. All results were reported correctly by the Xpert MRSA/SA Blood Culture test, except one specimen. The Xpert MRSA/SA Blood Culture test incorrectly identified one (1) SA strain (LGA251) as MSSA instead of MRSA. LGA251 contains a novel *mecA* gene representing a divergent *mecA* homologue *mecC* (i.e.,$mecA_{LGA251}$) located in a novel staphylococcal chromosome mec element, designated SCC *mec*type XI. The *mecA* primers and probes in the MRSA/SA Blood Culture test will not detect the *mecC* gene in this strain due to mutations in the primer/ probe binding regions. The *mecC* gene has substantially significant differences in homology when compared to the *mecA* gene in other non-variant MRSA strains.

16.3 Analytical Specificity (Exclusivity)

One hundred and one (101) organisms/strains were collected, quantitated, and tested using the Xpert MRSA/SA Blood Culture test. Of the 101 strains tested, 91 cultures were obtained from the American Type Culture Collection (ATCC); 1 was obtained from Culture Collection, University of Göteborg, Sweden (CCUG); 1 was obtained from Teruyo Ito, Juntendo University, Tokyo, Japan; 1 carbapenemase (KPC) producing *Klebsiella pneumoniae* strain was obtained from National Collection of Type Cultures (NCTC), UK; and 7 strains were obtained from the Network on Antimicrobial Resistance in SA (NARSA). These strains represent species phylogenetically related to SA or those potentially encountered in the hospital environment.

The organisms tested were identified as either Gram-positive (74), Gram-negative (24), or yeast (3). Methicillin-sensitive, coagulase-negative *Staphylococcus*, MSCoNS (27) and methicillin- resistant, coagulase-negative *Staphylococcus*, MRCoNS (12) were included. The organisms were further classified as either aerobic (94) or anaerobic (7).

Three 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**; **SA NEGATIVE**; none of the isolates were detected by the Xpert MRSA/SA Blood Culture test. The analytical specificity was 100%.

16.4 Interfering Substances Study

Substances that may be present in blood cultures and have potential to interfere with the Xpert MRSA/SA Blood Culture test were tested in the interfering substance study. Potentially interfering substances evaluated include, but are not limited to, anticoagulated whole blood with ACD, EDTA, Heparin, and Sodium Citrate, human plasma, three blood culture media bottles (Becton Dickinson BACTEC Plus Aerobic/F, BioMérieux BacT/ALERT SA (Standard Aerobic), and TREK Diagnostics VersaTREK REDOX1 (Aerobic), bilirubin, γ-globulin, hemoglobin, triglycerides, and sodium polyanetholesulfonate (SPS).

Bilirubin, γ-globulin, hemoglobin, and triglycerides were tested at concentrations approximately one log higher than reference levels. SPS was tested at a 10-fold higher concentration than found in blood culture media. Negative samples (n=8) were tested in each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n=8) were tested per substance with two clinical isolates each of MSSA (29213 and 102–04) and MRSA (SCC*mec* types II and III) spiked near the analytical LoD determined for each isolate. All results were compared to positive and negative buffer controls. All negative specimens were correctly reported **MRSA NEGATIVE; SA NEGATIVE** using the Xpert MRSA/SA Blood Culture test.

None of the potentially interfering substances had a statistically significant inhibitory effect on SPC performance in negative samples (p-value = >0.05). All of the positive MSSA specimens were correctly reported **MRSA NEGATIVE; SA POSITIVE** using the Xpert MRSA/SA Blood Culture test. All of the positive MRSA specimens were correctly reported **MRSA POSITIVE; SA POSITIVE** using the Xpert MRSA/SA Blood Culture test. None of the potentially interfering substances resulted in a Ct difference of ≥1 cycle relative to the buffer controls, and no false-negative results were reported.

16.5 Carry-Over Contamination Study

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples run following very high positive samples in the same GeneXpert module. This study consisted of a negative sample processed in the same GeneXpert module immediately following a very high positive sample ($6x10^7$ MSSA or MRSA cells) in the same GeneXpert Dx System module. This was repeated 40 times between 2 GeneXpert modules. A total of 84 runs per strain were tested (40 positive samples per system per strain and 44 negative samples per system per strain). There was no evidence of any carry-over contamination. All 40 MRSA positive samples were correctly reported **MRSA POSITIVE; SA POSITIVE**. All 40 MSSA positive samples were correctly reported **MRSA NEGATIVE; SA POSITIVE**. All 88 negative samples were correctly reported **MRSA NEGATIVE; SA NEGATIVE**.

16.6 Blood Culture Bottle Validation

The performance of the Xpert MRSA/SA Blood Culture test was evaluated with seven additional types of blood culture media. The following bottle types were evaluated for both MRSA and MSSA. See Table 6.

Table 6. Blood Culture Bottles

BDBACTEC™ PEDS PLUS™/F
BDBACTEC™ Plus Anaerobic/F
BD BACTEC™Standard Anaerobic/F
BD BACTEC™Standard/10 Aerobic/F
BDBACTEC™ LYTIC/10 Anaerobic/F
BacT/ALERT® SN standard anaerobic
VersaTREK™REDOX™ 2 (anaerobic)

Positive blood culture samples were created for each bottle type by adding negative human whole blood and one MRSA strain and one MSSA strain individually to a final bacterial concentration of 10 CFU/mL per bottle. The blood culture bottles were incubated until positive for growth. Upon reaching bottle positivity, an aliquot of each sample was tested at 1500 CFU/test in replicates of six for each bottle type. All positive replicates yielded the expected positive result for the targeted analytes present in the sample.

Negative blood culture samples were created for each bottle type by adding negative whole blood and incubating for 24 hours prior to testing with Xpert MRSA/SA Blood Culture test. All negative replicates yielded the expected negative result.

17 Reproducibility

Reproducibility of the Xpert MRSA/SA Blood Culture test was evaluated at three sites using samples comprised of cultured material spiked into a simulated matrix. The samples were prepared at concentration levels representing high negative (below LoD), low positive (~1X LoD) and moderate positive (~2-3X LoD) for both MRSA and MSSA. Two different strains of MRSA were used. Negative panel members were also included and were comprised of *Staphylococcus epidermidis* spiked into a simulated matrix. A panel of 11 samples was tested on five different days by two different operators three times per day at three sites (11 samples x 2 operators x 5 days x 3 replicates per day x 3 sites). One lot of Xpert MRSA/SA BC reagents was included in the study.

Xpert MRSA/SA Blood Culture tests were performed according to the Xpert MRSA/SA Blood Culture procedure. The rate of agreement for each panel member is presented in Table 7.

Table 7. Summary of Reproducibility Results – Agreement by Study Site/Instrument

Sample	Site 1/ GX Dx	Site 2 Inf-80	Site 3/Inf-48	% Total Agreement
MRSA-1 high neg (below LOD)	56.7% (17/30)	60.0% (18/30)	66.7% (20/30)	61.1% (55/90)
MRSA-1 low pos (~1X LOD)	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
MRSA-1 mod pos (~2-3X LOD)	100.0% (30/30)	100.0% (30/30)	100.0% (29/29)	100.0% (89/89)[a]
MRSA-2 high neg (below LOD)	43.3% (13/30)	53.3% (16/30)	70.0% (21/30)	55.6% (50/90)
MRSA-2 low pos (~1X LOD)	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
MRSA-2 mod pos (~2-3X LOD)	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
MSSA high neg (below LOD)	60.0% (18/30)	48.3% (14/29)	70.0% (21/30)	59.6% (53/89)[b]
MSSA low pos (~1X LOD)	96.7% (29/30)	100.0% (30/30)	96.7% (29/30)	97.8% (88/90)
MSSA mod pos (~2-3X LOD)	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
Negative-1	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)
Negative-2	100.0% (30/30)	100.0% (30/30)	100.0% (30/30)	100.0% (90/90)

[a] One sample indeterminate after initial and retest.

[b] One sample mistakenly not run.

The reproducibility of the Xpert MRSA/SA Blood Culture test was also evaluated in terms of the fluorescence signal expressed in cycle threshold (Ct) values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between- sites, between-lots, between-days, and between-runs for each panel member are presented in Table 8.

Table 8. Summary of Reproducibility Data

Target	Sample	Conc	Agree/ N	Agrmt (%)	Mean Ct	Between-Instrument		Between-Day		Between-Run[a]		Within-Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
spa	MRSA-1	high neg	55/90	61.1	35.6	0.18	0.5	0.21	0.6	0.00	0.0	0.95	2.7	0.99	2.8
	MRSA-1	low pos	90/90	100.0	32.8	0.27	0.8	0.00	0.0	0.00	0.0	0.62	1.9	0.67	2.1
	MRSA-1	mod pos	89/89	100.0	31.2	0.11	0.4	0.00	0.0	0.00	0.0	0.58	1.9	0.59	1.9
	MRSA-2	high neg	50/90	55.6	35.3	0.15	0.4	0.00	0.0	0.00	0.0	0.99	2.8	1.00	2.8
	MRSA-2	low pos	90/90	100.0	32.3	0.11	0.4	0.00	0.0	0.13	0.4	0.63	1.9	0.65	2.0
	MRSA-2	mod pos	90/90	100.0	30.7	0.00	0.0	0.00	0.0	0.00	0.0	0.55	1.8	0.55	1.8

Target	Sample	Conc	Agree/ N	Agrmt (%)	Mean Ct	Between-Instrument		Between-Day		Between-Run[a]		Within-Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
	MSSA	high neg	53/89	59.6	36.3	0.00	0.0	0.00	0.0	0.00	0.0	1.26	3.5	1.26	3.5
	MSSA	low pos	88/90	97.8	33.5	0.07	0.2	0.18	0.5	0.00	0.0	0.89	2.7	0.91	2.7
	MSSA	mod pos	90/90	100.0	31.7	0.08	0.2	0.20	0.6	0.17	0.6	0.48	1.5	0.56	1.8
	NEG-1	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
mec	MRSA-1	high neg	55/90	61.1	35.8	0.00	0.0	0.36	1.0	0.00	0.0	0.83	2.3	0.91	2.5
	MRSA-1	low pos	90/90	100.0	33.4	0.12	0.4	0.19	0.6	0.00	0.0	0.55	1.6	0.59	1.8
	MRSA-1	mod pos	89/89	100.0	31.9	0.08	0.2	0.00	0.0	0.00	0.0	0.46	1.4	0.47	1.5
	MRSA-2	high neg	50/90	55.6	35.8	0.00	0.0	0.34	0.9	0.00	0.0	1.03	2.9	1.08	3.0
	MRSA-2	low pos	90/90	100.0	32.8	0.11	0.3	0.00	0.0	0.16	0.5	0.51	1.6	0.54	1.7
	MRSA-2	mod pos	90/90	100.0	31.5	0.00	0.0	0.16	0.5	0.00	0.0	0.49	1.5	0.51	1.6
	MSSA	high neg	53/89	59.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	low pos	88/90	97.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	mod pos	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-1	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SCC	MRSA-1	high neg	55/90	61.1	37.2	0.20	0.5	0.37	1.0	0.35	1.0	0.82	2.2	0.98	2.6
	MRSA-1	low pos	90/90	100.0	34.5	0.19	0.5	0.23	0.7	0.00	0.0	0.59	1.7	0.66	1.9
	MRSA-1	mod pos	89/89	100.0	33.0	0.16	0.5	0.00	0.0	0.00	0.0	0.45	1.4	0.48	1.5
	MRSA-2	high neg	50/90	55.6	36.8	0.23	0.6	0.24	0.6	0.10	0.3	1.00	2.7	1.06	2.9
	MRSA-2	low pos	90/90	100.0	33.7	0.11	0.3	0.00	0.0	0.26	0.8	0.57	1.7	0.64	1.9
	MRSA-2	mod pos	90/90	100.0	32.4	0.00	0.0	0.09	0.3	0.00	0.0	0.45	1.4	0.46	1.4
	MSSA	high neg	53/89	59.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	low pos	88/90	97.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	mod pos	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-1	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SPC	MRSA-1	high neg	55/90	61.1	32.7	0.00	0.0	0.00	0.0	0.20	0.6	0.65	2.0	0.68	2.1
	MRSA-1	low pos	90/90	100.0	33.0	0.00	0.0	0.16	0.5	0.10	0.3	0.61	1.8	0.63	1.9
	MRSA-1	mod pos	89/89	100.0	33.0	0.27	0.8	0.00	0.0	0.00	0.0	0.83	2.5	0.87	2.6
	MRSA-2	high neg	50/90	55.6	33.1	0.23	0.7	0.00	0.0	0.10	0.3	0.85	2.6	0.89	2.7
	MRSA-2	low pos	90/90	100.0	32.9	0.15	0.5	0.00	0.0	0.00	0.0	0.78	2.4	0.79	2.4
	MRSA-2	mod pos	90/90	100.0	32.8	0.00	0.0	0.23	0.7	0.00	0.0	0.66	2.0	0.70	2.1
	MSSA	high neg	53/89	59.6	32.8	0.18	0.5	0.15	0.5	0.00	0.0	0.74	2.2	0.77	2.4

Target	Sample	Conc	Agree/ N	Agrmt (%)	Mean Ct	Between-Instrument		Between-Day		Between-Run[a]		Within-Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
	MSSA	low pos	88/90	97.8	32.9	0.00	0.0	0.00	0.0	0.00	0.0	0.72	2.2	0.72	2.2
	MSSA	mod pos	90/90	100.0	33.0	0.00	0.0	0.31	0.9	0.00	0.0	0.69	2.1	0.76	2.3
	NEG-1	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	90/90	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Agrmt=Agreement, Conc=Concentration, CV=coefficient of variation, N/A= Not Applicable for negative samples, SD=standard deviation.														

[a] A run is defined as the three samples per panel member run by one operator at one site on one day.

Note The variance estimate from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and CV is set to 0.

18 Instrument Systems Precision Study

An in-house precision study was conducted to compare the performance of the GeneXpert Dx, the Infinity-48 and the Infinity- 80 Instrument Systems using samples comprised of cultured material spiked into a simulated matrix. The samples were prepared at concentration levels representing high negative (below LoD), low positive (~1X LoD) and moderate positive (~2-3X LoD) for both MRSA and MSSA. Two different strains of MRSA were used. Negative panel members were also included and were comprised of *Staphylococcus epidermidis* spiked into a simulated matrix. A panel of 11 specimens was tested on 12 different days by two different operators four times per day per instrument (11 specimens x 2 operators x 12 days x 4 replicates per day x 3 instruments). One lot of Xpert MRSA/SA BC reagents was included in the study. Xpert MRSA/SA Blood Culture tests were performed according to the Xpert MRSA/SA Blood Culture test procedure. The rate of agreement for each panel member is presented in Table 9.

Table 9. Summary of Precision Results – Agreement by Instrument

Sample	GX Dx	Inf-48	Inf-80	% Total Agreement
MRSA-1 high neg (below LOD)	50.0% (48/96)	51.6% (49/95)	35.4% (34/96)	45.6% (131/287)[a]
MRSA-1 low pos (~1X LOD)	96.9% (93/96)	99.0% (95/96)	99.0% (95/96)	98.3% (283/288)
MRSA-1 mod pos (~2-3X LOD)	100.0% (96/96)	100.0% (96/96)	99.0% (95/96)	99.7% (287/288)
MRSA-2 high neg (below LOD)	80.2% (77/96)	78.1% (75/96)	80.2% (77/96)	79.5% (229/288)
MRSA-2 low pos (~1X LOD)	100.0% (96/96)	100.0% (96/96)	100.0% (96/96)	100.0% (288/288)
MRSA-2 mod pos (~2-3X LOD)	100.0% (96/96)	100.0% (96/96)	99.0% (95/96)	99.7% (287/288)
MSSA high neg (below LOD)	76.0% (73/96)	71.9% (69/96)	81.3% (78/96)	76.4% (220/288)
MSSA low pos (~1X LOD)	96.9% (93/96)	99.0% (95/96)	100.0% (96/96)	98.6% (284/288)

Sample	GX Dx	Inf-48	Inf-80	% Total Agreement
MSSA mod pos (~2-3X LOD)	100.0% (96/96)	100.0% (96/96)	100.0% (96/96)	100.0% (288/288)
Negative-1	100.0% (96/96)	100.0% (96/96)	100.0% (96/96)	100.0% (288/288)
Negative-2	100.0% (96/96)	100.0% (96/96)	100.0% (96/96)	100.0% (288/288)

[a] One sample was indeterminate after initial and retest.

The precision study results were also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-instruments, between-days, and between-runs for each panel member are presented in Table 10.

Table 10. Summary of Precision Data

Target	Sample	Conc	Agree/N	Agrmt (%)	Mean Ct	Between-Instrument		Between-Day		Between-Run[a]		Within-Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
spa	MRSA-1	high neg	131/287	45.6	34.4	0.00	0.0	0.00	0.0	0.00	0.0	1.09	3.2	1.09	3.2
	MRSA-1	low pos	283/288	98.3	32.9	0.02	0.1	0.16	0.5	0.00	0.0	0.78	2.4	0.80	2.4
	MRSA-1	mod pos	287/288	99.7	32.0	0.06	0.2	0.10	0.3	0.00	0.0	0.62	1.9	0.63	2.0
	MRSA-2	high neg	229/288	79.5	36.2	0.14	0.4	0.00	0.0	0.00	0.0	1.19	3.3	1.35	3.7
	MRSA-2	low pos	288/288	100.0	32.4	0.03	0.1	0.00	0.0	0.00	0.0	0.57	1.8	0.62	1.9
	MRSA-2	mod pos	287/288	99.7	31.1	0.12	0.4	0.00	0.0	0.00	0.0	0.49	1.6	0.51	1.7
	MSSA	high neg	220/288	76.4	36.4	0.21	0.6	0.00	0.0	0.00	0.0	1.36	3.7	1.59	4.4
	MSSA	low pos	284/288	98.6	33.8	0.09	0.3	0.18	0.5	0.00	0.0	0.87	2.6	0.90	2.7
	MSSA	mod pos	288/288	100.0	32.2	0.08	0.3	0.00	0.0	0.00	0.0	0.70	2.2	0.74	2.3
	NEG-1	Neg	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
mec	MRSA-1	high neg	131/287	45.6	34.5	0.00	0.0	0.11	0.3	0.00	0.0	0.86	2.5	0.87	2.5
	MRSA-1	low pos	283/288	98.3	33.4	0.07	0.2	0.14	0.4	0.00	0.0	0.61	1.8	0.63	1.9
	MRSA-1	mod pos	287/288	99.7	32.5	0.08	0.2	0.00	0.0	0.00	0.0	0.55	1.7	0.56	1.7
	MRSA-2	high neg	229/288	79.5	35.9	0.00	0.0	0.28	0.8	0.00	0.0	1.02	2.8	1.06	2.9
	MRSA-2	low pos	288/288	100.0	32.8	0.06	0.2	0.00	0.0	0.00	0.0	0.49	1.5	0.53	1.6
	MRSA-2	mod pos	287/288	99.7	31.5	0.14	0.5	0.05	0.2	0.00	0.0	0.45	1.4	0.47	1.5

Target	Sample	Conc	Agree/N	Agrmt (%)	Mean Ct	Between-Instrument		Between-Day		Between-Run[a]		Within-Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
	MSSA	high neg	220/288	76.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	low pos	284/288	98.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	mod pos	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-1	Neg	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SCC	MRSA-1	high neg	131/287	45.6	36.7	0.18	0.5	0.00	0.0	0.00	0.0	1.51	4.1	1.52	4.1
	MRSA-1	low pos	283/288	98.3	34.7	0.00	0.0	0.20	0.6	0.00	0.0	1.11	3.2	1.13	3.2
	MRSA-1	mod pos	287/288	99.7	33.7	0.12	0.3	0.00	0.0	0.00	0.0	0.78	2.3	0.78	2.3
	MRSA-2	high neg	229/288	79.5	37.3	0.00	0.0	0.32	0.8	0.00	0.0	1.03	2.8	1.17	3.1
	MRSA-2	low pos	288/288	100.0	34.2	0.02	0.1	0.00	0.0	0.00	0.0	0.44	1.3	0.50	1.5
	MRSA-2	mod pos	287/288	99.7	33.0	0.12	0.4	0.03	0.1	0.00	0.0	0.49	1.5	0.50	1.5
	MSSA	high neg	220/288	76.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	low pos	284/288	98.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSSA	mod pos	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-1	Neg	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NEG-2	Neg	288/288	100.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
SPC	MRSA-1	high neg	131/287	45.6	33.4	0.00	0.0	0.17	0.5	0.00	0.0	0.84	2.5	0.86	2.6
	MRSA-1	low pos	283/288	98.3	33.4	0.10	0.3	0.21	0.6	0.00	0.0	0.77	2.3	0.80	2.4
	MRSA-1	mod pos	287/288	99.7	33.4	0.08	0.2	0.15	0.5	0.00	0.0	0.72	2.2	0.74	2.2
	MRSA-2	high neg	229/288	79.5	33.4	0.00	0.0	0.00	0.0	0.00	0.0	0.82	2.4	0.82	2.4
	MRSA-2	low pos	288/288	100.0	33.4	0.02	0.1	0.00	0.0	0.00	0.0	0.73	2.2	0.77	2.3
	MRSA-2	mod pos	287/288	99.7	33.3	0.00	0.0	0.09	0.3	0.00	0.0	0.74	2.2	0.75	2.2
	MSSA	high neg	220/288	76.4	33.4	0.00	0.0	0.20	0.6	0.00	0.0	0.83	2.5	0.85	2.6
	MSSA	low pos	284/288	98.6	33.5	0.00	0.0	0.00	0.0	0.00	0.0	0.86	2.6	0.87	2.6
	MSSA	mod pos	288/288	100.0	33.1	0.11	0.3	0.00	0.0	0.00	0.0	0.75	2.2	0.77	2.3
	NEG-1	Neg	288/288	100.0	33.4	0.00	0.0	0.13	0.4	0.00	0.0	0.85	2.6	0.87	2.6
	NEG-2	Neg	288/288	100.0	33.5	0.00	0.0	0.02	0.1	0.00	0.0	0.84	2.5	0.84	2.5

Target	Sample	Conc	Agree/N	Agrmt (%)	Mean Ct	Between-Instrument		Between-Day		Between-Run[a]		Within-Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
	Agrmt=Agreement, Conc=concentration, CV=coefficient of variation, N/A=Not Applicable for negative samples, SD=standard deviation.														

[a] A run is defined as the four samples per panel member run by one operator at one site on one day.

Note The variance estimate from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, the variability as measured with SD and CV is set to 0.

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20 Cepheid Headquarters Locations

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21 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

United States

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France

Telephone: + 33 563 825 319
Email: support@cepheideurope.com

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

22 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
	Expiration date
	Temperature limitation
	Biological risks
	Warning
	Authorized Representative in Switzerland
	Importer

Cepheid AB
Röntgenvägen 5
SE-171 54 Solna,
Sweden

Cepheid Switzerland GmbH
Zürcherstrasse 66
Postfach 124, Thalwil
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23 Revision History

Section	Description of Change
Table of Symbols	Added CH REP and Importer symbols and definitions to Table of Symbols. Added CH REP and Importer information with Switzerland address.
Revision History	Updated revision history table.