



## BD GeneOhm™ StaphSR Assay



IVD

<b>USA</b>	<b>REF 441449</b>	200 Tests
	<b>REF 441448</b>	48 Tests
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<b>TAIWAN</b>	<b>REF 441253</b>	200 Tests
	<b>REF 441252</b>	48 Tests

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## English

### Intended use

The BD GeneOhm™ StaphSR Assay is a qualitative *in vitro* diagnostic test for the rapid detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) directly from positive blood culture. The assay utilizes polymerase chain reaction (PCR) for the amplification of specific targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed on Gram positive cocci, identified by Gram stain, from positive blood cultures. The BD GeneOhm™ StaphSR Assay is not intended to monitor treatment for MRSA/SA infections. Subculturing of positive blood cultures is necessary for further susceptibility testing.

### Summary and explanation of the test

To test a positive blood culture, an aliquot of the culture media is transferred into a sample buffer tube and lysed. An aliquot of the lysate is added to a mix of PCR reagents which contains the *S. aureus*- and MRSA-specific primers used to amplify the genetic targets, if present. The assay also includes an internal control (IC) to detect PCR inhibitory specimen and to confirm the integrity of assay reagents. Amplified targets are detected with hybridization probes labeled with quenched fluorophores (molecular beacon probes). Amplification, detection, and signal interpretation are done automatically by the Cepheid SmartCycler® software. The whole procedure takes about 60 to 75 minutes, depending on the number of specimens processed. Recovery of organisms for epidemiological typing or for further antibiotic susceptibility testing can be done by inoculating appropriate culture medium during specimen preparation or up to 3 days after blood bottle positivity.

*S. aureus* is a major cause of nosocomial infections with clinical manifestations ranging from pustules to sepsis and death<sup>1</sup>. In the laboratory, *S. aureus* is mainly isolated from nares, skin, wounds, and blood cultures. In a hospital setting, *S. aureus* is usually transmitted from patient to patient through the contaminated hands of healthcare workers.

Treatment of *S. aureus* infections has become a real challenge with the emergence of strains resistant to previously effective antimicrobial agents. Methicillin-resistant strains of *S. aureus* are frequently encountered in health-care settings, and represent nearly 60% of isolates from hospital acquired *S. aureus* in some North American hospitals<sup>2</sup>. Risk factors for infection with MRSA in healthcare settings include prolonged hospital stay, proximity to patients infected with MRSA, exposure to multiple and prolonged broad-spectrum antibiotic treatments, and MRSA carriage.

*S. aureus* is responsible for up to 25% of bloodstream infections<sup>3,4</sup>, among which, 26 to 47% are caused by MRSA<sup>3,5</sup>. Bacteremia due to *S. aureus* have been associated with mortality rates ranging from 25%-35%<sup>4,6</sup>, emphasizing the importance in reducing the time-to-result in order to help prevent patient death and decrease costs related to prolonged hospital stays.

Traditional techniques used for the detection of *S. aureus* and MRSA require culture steps and isolation of pure colonies, followed by agglutination testing to identify *S. aureus* and either oxacillin susceptibility testing, detection of the *mecA* gene, or detection of the penicillin binding protein (PBP 2a) to identify MRSA. Once a blood culture is declared positive, a minimum of 16 hours is required to resolve the *S. aureus* and MRSA status, with a median time of more than 48 hours, when using these conventional methods.

With the rapidity at which *S. aureus* infections can spread, and the morbidity and mortality associated with these infections, the capability of detecting *S. aureus* and MRSA within hours of a blood culture becoming positive represents a definite improvement over current practices. A better time-to-result enables a faster diagnosis and allows for more effective patient treatment and management.

### Principle of the procedure

Following specimen lysis, amplification of the targets occurs if either or both are present [MRSA: sequence near the insertion site of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*); *S. aureus* (SA): another *S. aureus* specific sequence, unattached to the SCC*mec* cassette]. Amplification of the IC also takes place unless PCR inhibitory substances are present.

The amplified DNA targets are detected with molecular beacon probes, hairpin-forming single-stranded oligonucleotides labelled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the absence of target, the fluorescence is quenched. In the presence of target, the hairpin structure opens upon beacon/target hybridization, resulting in emission of fluorescence. For the detection of MRSA amplicon, the molecular beacon probe contains the fluorophore FAM at the 5' end and the non-fluorescent quencher moiety DABCYL at the opposite 3' end of the oligonucleotide. For the detection of *S. aureus* amplicon, the molecular beacon probe is labelled with the fluorophore TexasRed at the 5' end and the quencher DABCYL at the 3' end. For the detection of the IC amplicon, the molecular

beacon probe contains the fluorophore TET at the 5' end and the quencher DABCYL at the 3' end. Each beacon-target hybrid fluoresces at a wavelength characteristic of the fluorophore used in the particular molecular beacon probe. The amount of fluorescence at any given cycle, or following cycling, depends on the amount of specific amplicon present at that time. The SmartCycler software simultaneously monitors the fluorescence emitted by each molecular beacon probe, interprets all data, and provides a final result at the end of the cycling program (see Interpretation of Results).

## Reagents

<b>BD GeneOhm™ StaphSR Assay</b>	<b>48 Tests</b>	<b>200 Tests</b>
<b>Sample Buffer</b>	<b>60 X 1 mL</b>	<b>240 X 1 mL</b>
Tris-EDTA buffer		
<b>Lysis tube</b>	<b>50 tubes</b>	<b>200 tubes</b>
Glass beads		
<b>Master Mix (8 reactions each)</b>	<b>8 tubes</b>	<b>34 tubes</b>
< 0.0005% DNA polymerase complex		
< 0.001% Internal control - non-infectious DNA containing MRSA-primer binding sequences and a unique sequence for probe hybridization		
< 0.002% primers		
< 0.002% molecular beacon probes		
< 0.05% dATP, dCTP, dGTP, dTTP		
Bovine serum albumin		
Carbohydrate		
MgCl <sub>2</sub>		
< 0.001% non-infectious <i>Staphylococcus epidermidis</i> genomic DNA (ATCC 14990)		
<b>Control DNA</b>	<b>8 tubes</b>	<b>34 tubes</b>
Tris-EDTA buffer		
Carbohydrate		
< 0.001% non-infectious genomic MRSA DNA (ATCC 43300)		
<b>Diluent</b>	<b>8 X 700 µL</b>	<b>34 X 700 µL</b>
Tris-HCl buffer		
MgCl <sub>2</sub>		
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		
KCl		

## Precautions

- Do not use the kit if the outer carton safety seal is broken.
- Do not use reagents if their protective pouches are open or torn upon arrival.
- Close protective pouches of Master Mix and Control DNA quickly with the zip seal after each use.
- Do not remove desiccant from Master Mix and Control DNA pouches.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents when removing aliquots from tubes. The use of sterile DNase-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- To avoid contamination of the environment with *S. aureus* and MRSA amplicons, do not open the reaction tubes post-amplification.
- Use a new pipettor tip for each specimen or reagent.

- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- In cases where open-tube PCR tests are conducted in the same general area by the laboratory, separated and segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating lyophilized reagents.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*<sup>7</sup> and in CLSI Document M29<sup>8</sup>.
- Wear protective clothing and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.
- Do not pipet by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

## Materials provided

- Sample Buffer
- Lysis tube
- Master Mix
- Control DNA
- Diluent
- SmartCycler reaction tubes, 25 µL capacity
- Specimen identification labels

## Storage, handling and stability

### Collected specimens

#### Positive Blood Cultures

Positive blood culture specimens can be stored for up to 3 days at room temperature (15-25°C) before testing.

If positive blood cultures require shipment for secondary analysis, they should be kept between 2°C and 30°C during transport. Protect against freezing or exposure to excessive heat.

Positive blood culture specimens that can be tested within 18 hours can be kept at temperatures up to 35°C.

#### Lysates (DNA extracts)

Internal storage studies demonstrated that lysates can be used for testing after two years when stored at -20°C.

## Reagents

**Note:** Storage conditions must follow the specifications written on each pouch.

Kit Component		Master Mix and Control DNA (white and red strip labels)	Lysis tube (yellow cap)	Sample Buffer and Diluent (blue cap and black strip label respectively)
Sealed pouch	Temperature	2-25 °C	2-25 °C	2-25 °C
	Stability	Expiration date	Expiration date	Expiration date
Opened pouch	Temperature	2-8 °C <sup>1</sup>	2-25 °C <sup>2,3</sup>	2-25 °C <sup>2,3</sup>
	Stability	1 month <sup>3</sup>	Expiration date <sup>3</sup>	2 months <sup>3</sup>

<sup>1</sup> Once the original seal on the pouch is broken, carefully close the pouch with the zip seal after each use and store at appropriate temperature.

<sup>2</sup> Although these reagents can be stored at room temperature, they should be kept with their accompanying reagents of the same lot at 2-8 °C.

<sup>3</sup> Provided the bag is properly closed with the zip seal after each use.

Kit Components outside of their protective pouches			Master mix and Control DNA (white and red strip labels)
Not reconstituted tubes	Temperature		15-25 °C
	Stability		2 hours <sup>1</sup>
Reconstituted tubes <sup>1</sup>	Original container	Temperature	2-8 °C
		Stability	3 hours <sup>1</sup>
	SmartCycler tube	Temperature	2-8 °C
		Stability	45 minutes <sup>1</sup>

<sup>1</sup> Discard unused tubes after expiration of indicated stability.

## Materials required but not provided

- **Blood culture bottles for Bactec™ system (Becton Dickinson):**
  - BD Bactec™ Plus Aerobic/F catalog no 442192
  - BD Bactec™ Plus Anaerobic/F Culture Vials catalog 442193
  - BD Bactec™ Lytic/10 Anaerobic/F Culture Vials catalog no 442265
- **Or blood culture bottles for BacT/Alert® system (Biomérieux):**
  - BacT/Alert® SA (Standard Aerobic) catalog no 259789
  - BacT/Alert® SN (Standard Anaerobic) catalog no 259790
  - BacT/Alert® FA (FAN Aerobic) catalog no 259791
  - BacT/Alert® FN (FAN Anaerobic) catalog no 259793
- **Gram staining reagents**
- BBL™ CHROMagar™ Staph aureus catalog no. 214982, Mannitol Salt Agar (MSA) catalog no. 221173 or 221271 or equivalent media (optional),
- 5% sheep blood agar plate (eg. BBL™ Trypticase Soy Agar (TSA II) with 5% Sheep Blood, BD catalog no. 221239 or 221261) (optional)
- **Vortex** Genie 2 (Fisher) with 1.5 mL microtube holder or equivalent; for processing multiple samples, an adapter capable of holding multiple tubes can be used
- **Micropipettors** (accurate range between 1-10 µL, 10-100 µL and 100-1000 µL)
- **Sterile DNase-free** filter-blocked or positive displacement **micropipettor tips**
- DNase free microcentrifuge tubes
- Disposable **gloves**, powderless
- **Microcentrifuge** for low speed centrifugation
- **Dry heating block** specific for 1.5 mL tubes or water bath
- Ice or **cooling block** specific for 1.5 mL tubes

- **Cap removal tool** (e.g. MATRIX catalog no. 4469) (optional)
- Stopwatch or **timer**
- **SmartCycler starter system with Dx Software** (processing block, user manual, accessory kit, and computer)

## Instructions for use

### Specimen collection – positive blood culture

In order to obtain an adequate specimen, the procedure for specimen collection must be followed carefully.

**Using a recommended blood culture bottle** (refer to “Materials required but not provided”):

1. Collect and process blood specimens following manufacturer instructions and hospital standard operating procedures.
2. Label and transport inoculated blood bottles according to manufacturer instructions and hospital standard operating procedures.
3. Incubate blood culture bottles using an appropriate automated blood culture system.
4. Upon positivity determination, remove blood culture bottles from incubation and refer to the section entitled “Storage, handling and stability – Collected specimens for storage and handling.”
5. Perform a Gram Stain from the positive blood culture and go to “specimen preparation” for Gram Positive cocci results. For results other than Gram positive cocci, refer to “culturing of specimens.”

**NOTE: In a mixed culture containing Gram positive cocci and other organisms (e.g. Gram negative bacilli, yeast), results can be false negative or variable depending on the concentration of Gram positive cocci present, particularly if the concentration of Gram positive cocci is below the LOD of the assay. When mixed culture is observed in the Gram stain, it is recommended that users follow the recommendations under “culturing of specimens.”**

6. Concomitant cultures are necessary for recovery and identification of other organisms observed in the Gram stain, or for further susceptibility testing and/or epidemiological typing of *Staphylococcus aureus* (SA) or methicillin-resistant *Staphylococcus aureus* (MRSA).

### BD GeneOhm™ StaphSR assay specimen preparation

**Note:** One Sample Buffer tube (**blue cap**) and one Lysis tube (**yellow cap**) are required for each specimen to be tested. Remove the required number of tubes from their protective pouches, **remove the excess air, and close the pouches quickly with the zip seal.**

For culturing specimens prior to performing the BD GeneOhm™ StaphSR assay, refer to section “Culturing of specimens - Streak-plate method for details.”

#### Cell suspension

1. **Transfer 2 µL of homogeneous positive blood culture to a Sample Buffer tube (blue cap).**  
Identify the sample buffer tube on the cap and/or the tube label.
2. **Vortex at high speed for 15 seconds.**  
For processing multiple samples, an adapter capable of holding multiple tubes can be used.
3. **Transfer 50 µL of cell suspension to a lysis tube (yellow cap); close tightly.**  
Use a new pipettor tip for each specimen.

#### Lysis

1. **Vortex the Lysis tube containing the cell suspension for 5 minutes at high speed.**  
For processing multiple samples, an adapter capable of holding multiple tubes can be used.
2. **Centrifuge the Lysis tube briefly (quick spin).**  
At low speed for 2 to 5 seconds; to bring the contents to the bottom of the tube.
3. **Heat at 95 ± 2 °C for two (2) minutes.**  
Use a dry heating block specific for 1.5 mL tubes, or a water bath.
4. **Keep the Lysis tube on ice or on a cooling block.**  
If the lysates cannot be used for immediate testing, store them at -20 ± 5 °C for later use.

## **BD GeneOhm™ StaphSR assay procedure**

**Note:** One reconstituted Master Mix tube (**white label**) will yield enough reagents to run 8 reactions. Allow one SmartCycler tube per specimen to be tested and 2 additional SmartCycler tubes for the positive and the negative control. **One (1) positive and one (1) negative control must be included in each BD GeneOhm™ StaphSR assay run. One Control DNA (red strip label) is required per assay run. One Diluent tube (black strip label) is required for the preparation of up to 3 master mix tubes. Remove the required number of tubes from their protective pouches, remove the excess air, and close the pouches quickly with the zip seal. Prepare only enough SmartCycler tubes to fill available I-CORE® modules on the SmartCycler instrument.**

- 1. Place the required number of Master Mix tube(s) on ice or on a cooling block specific for 1.5 mL tubes.**
- 2. Add 225 µL of Diluent (black strip label) to each Master Mix tube.**

Insert the micropipettor tip through the septum of the cap of the Master Mix tube. Do not insert the tip too deeply into the cap. Deliver the diluent. Discard the unused diluent afterwards.
- 3. Vortex the tube(s) for 5-10 seconds.**

Place the tube on ice or on a cooling block specific for 1.5 mL tubes until ready for use (reconstituted Master Mix tubes are stable for 3 hours on ice or on a cooling block).
- 4. Place a Control DNA tube (red stripe label) on ice or on a cooling block specific for 1.5 mL tubes.**
- 5. Add 225 µL of Sample Buffer (blue cap) to the Control DNA tube.**

Insert the micropipettor tip through the septum of the cap of the Control DNA tube. Do not insert the tip too deeply into the cap. Deliver the Sample Buffer.
- 6. Vortex the tube for 5-10 seconds.**

Place the tube on ice or on a cooling block specific for 1.5 mL tubes until ready to use.
- 7. Place the required number of SmartCycler tubes on the SmartCycler cooling block.**

Allow one SmartCycler tube per specimen and two more SmartCycler tubes for the controls. **Avoid touching the optical detection windows at the bottom edges of the tube as well as the lower diamond-shaped area.**

**The following steps MUST be performed WITHIN FORTY FIVE MINUTES:**

- 8. Add 25 µL of reconstituted Master Mix to the SmartCycler tubes (appropriate pipetting technique required to ensure proper transfer of the solution).**

Remove the cap before pipetting the reagent. Deliver the liquid into the reservoir (upper part) of the SmartCycler tubes. Identify the SmartCycler tubes on the cap. Specimen identification labels can be used (provided with the kit). Discard the unused Master Mix.
- 9. Add 3.0 µL of each lysed specimen to a different SmartCycler tube previously filled with reconstituted Master Mix; close the tubes.**

Be careful not to aspirate beads when pipetting from the Lysis tube. After addition of the specimen, pipet up and down 2-3 times in the reservoir to ensure transfer of the complete volume. Use a new micropipettor tip for each specimen.
- 10. Add 3.0 µL of the reconstituted Control DNA to the next to last SmartCycler tube (Positive Control); close the tube.**

After addition of the DNA, pipet up and down 2-3 times in the reservoir to ensure transfer of the complete volume. Identify as the positive control. Discard the unused Control DNA.
- 11. Add 3.0 µL of Sample Buffer (blue cap) to the last SmartCycler tube (Negative Control); close the tube.**

Use the Sample Buffer tube from step 5. This will monitor for PCR contamination that might occur during manipulation of specimens. Identify as negative control. Discard the unused Sample Buffer afterward.
- 12. Centrifuge all reaction tubes for 5-10 seconds.**

Use the specially adapted microcentrifuge provided with the SmartCycler instrument.
- 13. Keep the tubes at 2-8 °C on the SmartCycler cooling block before loading on the instrument.**

The remaining lysates should be frozen at -20 ± 5 °C for later use, if necessary.

**14. Create a run with the BD GeneOhm™ StaphSR assay protocol.**

Refer to the SmartCycler Dx Software Operator Manual if needed. Enter the identification parameters for the specimens before starting the run.

**15. Insert each reaction tube into an I-CORE module of the SmartCycler and close the I-CORE lid.**

Place the positive and negative controls at their appropriate position (see the section entitled "Quality control"). Press all the tubes firmly down into place.

**16. Start the run.****Culturing of specimens - streak-plate method**

To perform antimicrobial susceptibility testing, epidemiological typing, or identification of species other than *S. aureus*, use the streak-plate method outlined below.

1. When Gram positive cocci are observed from positive blood culture, inoculate a blood agar plate by transferring a few drops of the positive blood culture onto the first quadrant of the plate. This may also be performed if a mixed culture is observed.
2. With a sterile inoculating loop, streak the inoculum into the remaining quadrants of the blood agar plate.
3. Incubate the plate for 24-48 hours at 35 °C.
  - For mixed culture, isolate Gram positive colonies and dilute in saline to a turbidity of 0.5 McFarland (~1.5 X 10<sup>8</sup> CFU/mL). Process and test as a specimen.
  - For pure culture, identify and confirm *S. aureus* colonies and perform antimicrobial susceptibility testing according to standard methods.

**Quality control****Assay (positive and negative) controls**

Quality control procedures are designed to monitor assay performance. The positive control is intended to monitor substantial reagent failure. The negative control is used to detect reagent or environmental contamination (or carry-over) by either *S. aureus* or MRSA DNA or amplicons. Positive and negative controls are assay controls (run controls). An invalid control invalidates the run.

One positive control and one negative control must be included in each assay run on the SmartCycler. The software automatically assigns the position of the controls on the instrument (refer to the SmartCycler Dx Software Operator Manual).

**Specimen processing (positive and negative) controls**

Additional control strains may be tested according to guidelines or requirements of local, state and/or federal regulations or accreditation organizations. A reference MRSA strain (e.g. American Type Culture Collection, ATCC 43300), a well characterized MRSA clinical isolate, a reference MSSA strain (e.g. ATCC 25923) or a well characterized clinical specimen may be used as a specimen processing control; MRSA type iii and vii strains, if available, may be used as additional positive controls to monitor assay probes and primers not directly controlled in the assay; while any other non-*Staphylococcus aureus* (e.g. *Staphylococcus epidermidis* ATCC 14990) may be used as an external negative control.

Resuspend isolated colonies from an 18- to 24-h 5% sheep blood agar plate in saline to a turbidity of 0.5 McFarland (~1.5 X 10<sup>8</sup> CFU/mL). Process and test as a specimen (refer to the sections entitled "Specimen preparation" and "BD GeneOhm™ StaphSR assay procedure"), including controls. All specimens and controls should yield valid results (no invalid positive or negative control; no failed internal control; and no incorrect specimen processing control results - when specimen processing controls are performed). In the event of an incorrect specimen processing control result, it is recommended that new specimens (aliquots) be obtained from the positive blood cultures, and that these specimens be retested along with new controls before reporting results.

For general QC guidance, the user may wish to refer to CLSI C24<sup>9</sup>.

## Interpretation of results

The decisional algorithm for the BD GeneOhm™ StaphSR assay is embedded in the SmartCycler software. The interpretation of assay results is done according to the following criteria:

Assay result reported	IC result reported	Interpretation of result
NEG	PASS	No <i>S. aureus</i> DNA detected
POS	NA	MRSA DNA detected
Reactive	NA	<i>S. aureus</i> DNA detected, no MRSA DNA detected
Unresolved	FAIL	Unresolved—inhibitory specimen or reagent failure
ND	ND	Not determined due to I-CORE Module failure (with Warning or Error Codes <sup>1</sup> )

IC - Internal Control; NA – not applicable; ND – not determined

<sup>1</sup> Refer to the SmartCycler Dx Software Operator Manual for interpretation of warning and error codes.

**An invalid positive or negative control invalidates the assay run.** In such cases, assay results obtained for that run are invalid and must not be reported. Invalid assay runs, instrument error codes, and instrument warnings are flagged on-screen and on reports. Before reporting results, always verify that the assay run is valid.

Refer to the SmartCycler Dx Software Operator Manual for printing of results.

### **Invalid Assay Run**

Using each frozen specimen lysate, prepare new reaction tubes for all specimens within that assay run along with new control tubes.

### **Unresolved specimen**

Repeat testing with the corresponding frozen specimen lysate. The effect of the freeze-thaw cycle has been shown to reduce PCR inhibitory substances.

In the event that repeat testing is still unresolved (and in a valid run), this final result remains non-reportable. In such cases, process and test a new specimen sample from the positive blood culture, along with new control tubes.

### **Specimen not determined due to I-CORE module failure**

Repeat testing with the corresponding frozen specimen lysate. For the interpretation of warning or error code messages, refer to the SmartCycler Dx Software Operator Manual.

## Limitations of the procedure

- Sufficient pediatric samples were not analyzed during the clinical investigation, therefore, the performance of this assay with pediatric samples is unknown.
- Negative MRSA or Staphylococcus aureus result should not be used as the sole basis for diagnosis, treatment, or management decisions.
- The performance characteristics of this assay have not been established with automated real-time PCR instruments other than the SmartCycler instrument.
- The use of blood culture bottle types other than those listed in the “Materials required but not provided” section is not recommended. This test is for use only with positive blood culture bottle specimens; performance characteristics of other clinical specimen types have not been established.
- Negative test results may occur from improper specimen collection, handling or storage, presence of inhibitor, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. In a mixed culture containing Gram positive cocci and other organisms (e.g. Gram negative bacilli, yeast), results can be false negative or variable depending on the concentration of Gram positive cocci present. Careful compliance with the instructions given in this insert and in the SmartCycler Dx Software Operator Manual is necessary to avoid erroneous results. Use of this test should be limited to personnel trained on the procedure and on the use of the SmartCycler.

- Although there is no need for reagent preparation and the main technical operation is pipetting, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.
- BD GeneOhm™ StaphSR assay results may sometimes be unresolved, not determined (due to I-CORE module failure) or invalidated by an invalid control. Retesting will be required which can lead to delays in obtaining assay results.
- A positive test result does not necessarily indicate the presence of viable organisms. It is however presumptive for the presence of *Staphylococcus aureus* or MRSA. For MRSA detection, the BD GeneOhm™ StaphSR assay simultaneously targets the SCCmec cassette (carrying the *mecA* gene) and a *S. aureus* specific sequence located within the *orfX* gene. The BD GeneOhm™ StaphSR assay does not detect the *mecA* gene directly nor the penicillin binding protein (PBP 2a) encoded by this gene. For *S. aureus* detection, the BD GeneOhm™ StaphSR assay directly targets a sequence specific to *S. aureus*.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MRSA/SA variants resulting in a false negative result with the BD GeneOhm™ StaphSR assay.
- Genetic rearrangements, translocations, or deletions of the SCCmec cassette in some MRSA strain variants may result in false positive results.
- In a mixed culture containing both MRSA and MSSA, the LOD of MRSA can be variable (up to 30 copies/reaction instead of 15 copies/reaction) when extremely high concentrations of MSSA are present (e.g. a ratio of MRSA:MSSA of 1:500 or 1:1000).
- Preparation of Master Mix and positive and negative controls must be done in an environment that does not exceed 25°C.

## Performance characteristics

### Clinical performance

Performance characteristics of the BD GeneOhm™ StaphSR assay were determined in a multi-site prospective investigational study. Five medical centers, two in Canada and three in the U.S., having Methicillin-resistant *Staphylococcus aureus* (MRSA) culture-based methods already in place, participated in the study. To be enrolled in the study, patients had to provide informed consent and be eligible for Methicillin-resistant *Staphylococcus aureus* testing according to hospital policies. Screening criteria included, but were not limited to: positive blood culture obtained with a BD BACTEC™ or a BacT/Alert® blood culture bottle.

The reference culture method was performed on positive blood bottles presenting growth of a Gram positive cocci bacteria within 36 hours of being declared positive by the instrument. The reference method consisted of an initial analysis on a blood agar plate after an overnight incubation. Presumptive colonies of *S. aureus* were confirmed with either a latex agglutination assay, tube coagulase testing, or an automated identification system. Confirmed colonies were tested for methicillin resistance using oxacillin screen agar, PBP2<sup>1</sup> latex agglutination test, or an automated antimicrobial susceptibility testing system using appropriate panels. Plates were stored at 2-8°C for further testing, if required. An MRSA and/or *S. aureus* culture-positive specimen was defined as a specimen positive for MRSA and/or *S. aureus* by the culture technique. An MRSA or *S. aureus* culture-negative specimen was defined as a specimen negative for MRSA and *S. aureus* by the culture technique.

In total, 1183 positive blood bottles were found compliant and were screened for *S. aureus* and methicillin-resistant *S. aureus* with the culture method of reference described above and the BD GeneOhm™ StaphSR assay (Table 1). In comparison to the reference culture methods, the BD GeneOhm™ StaphSR assay identified 100% of the MRSA positive specimens and 98.8 to 100% of the *S. aureus* positive specimens (Tables 2 and 3).

**Table 1. Results obtained with BD GeneOhm™ StaphSR for MRSA and *S. aureus* in comparison to the reference methods.**

	<b>MRSA</b>	<b><i>S. aureus</i></b>																																
<b>Site 1</b>																																		
	<b>Culture/ID-AST System 1</b>	<b>Culture/ID-AST System 1</b>																																
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**Table 2. Performance obtained with BD GeneOhm™ StaphSR for MRSA (by investigational site) in comparison to the reference methods.**

Site	MRSA prevalence	MRSA Positive % Agreement (95% CI) <sup>1</sup>	MRSA Negative % Agreement (95% CI) <sup>1</sup>	Overall % Agreement
Site 1	13.7% (61/446)	100%(61/61) (94.1%-100%)	98.7% (380/385) (97.0% - 99.6%)	98.9%
Site 2	18.0% (24/133)	100%(24/24) (85.8%-100%)	98.2% (107/109) (93.5% - 99.8%)	98.5%
Site 3	9.1% (21/232)	100%(21/21) (83.9%-100%)	100.0% (211/211) (98.3% - 100.0%)	100%
Site 4	16.8% (48/286)	100% (48/48)(92.6%-100%)	98.3% (234/238) (95.8% - 99.5%)	98.6%
Site 5	2.3% (2/86)	100%(2/2) (15.8%-100%)	100.0% (84/84) (95.7% - 100.0%)	100%

<sup>1</sup> Binomial 95% exact confidence intervals.**Table 3. Performance obtained with BD GeneOhm™ StaphSR for *S. aureus* (by investigational site) in comparison to the reference methods.**

Investigational site	<i>S. aureus</i> prevalence	<i>S. aureus</i> Positive % Agreement (95% CI) <sup>1</sup>	<i>S. aureus</i> Negative % Agreement (95% CI) <sup>1</sup>	Overall % Agreement
Site 1	22.2% (99/446)	100.0% (99/99) (96.3%-100%)	100% (347/347)(98.9%-100%)	100%
Site 2	30.1% (40/133)	100% (40/40)(91.2%-100%)	98.9% (92/93) (94.2%-100%)	99.2%
Site 3	35.8% (83/232)	100% (83/83)( 95.7%-100%)	100% (149/149) (97.6%-100%)	100%
Site 4	29.7% (85/286)	98.8% (84/85) (93.6% - 100.0%)	96.5% (194/201) (93.0% - 98.6%)	97.2%
Site 5	9.3% (8/86)	100% (8/8) (63.1% - 100.0%)	100% (78/78) (95.4%-100%)	100%

<sup>1</sup> Binomial 95% exact confidence intervals.**Table 4. Unresolved results**

Investigational site	% Initial Unresolved with 95% exact confidence intervals		% Repeat Unresolved with 95% exact confidence intervals	
Site 1	0.0% (0/446)	(0.0% - 0.8%)	0.0% (0/446)	(0.0% - 0.8%)
Site 2	0.0% (0/133)	(0.0% - 2.7%)	0.0% (0/133)	(0.0% - 2.7%)
Site 3	0.0% (0/232)	(0.0% - 1.6%)	0.0% (0/232)	(0.0% - 1.6%)
Site 4	0.3% (1/286)	(0.0% - 1.9%)	0.0% (0/286)	(0.0% - 1.3%)
Site 5	0.0% (0/86)	(0.0% - 4.2%)	0.0% (0/86)	(0.0% - 4.2%)

**Table 5. Invalid assays**

Investigational site	% Initial invalid runs with 95% exact confidence intervals		% Invalid repeat runs with 95% exact confidence intervals	
Site 1	1.8% (2/113)	(0.2% - 6.2%)	0.0% (0/113)	(0.0% - 3.2%)
Site 2	8.9% (5/56)	(3.0% - 19.6%)	0.0% (0/56)	(0.0% - 6.4%)
Site 3	2.9% (2/69)	(0.4% - 10.1%)	0.0% (0/69)	(0.0% - 5.2%)
Site 4	2.4% (2/84)	(0.3% - 8.3%)	0.0% (0/84)	(0.0% - 4.3%)
Site 5	7.7% (5/65)	(2.5% - 17.0%)	0.0% (0/65)	(0.0% - 5.5%)

### **Analytical Specificity**

Genomic DNA from 66 non-Staphylococcal strains (representing 64 species), along with genomic DNA from 33 coagulase-negative *Staphylococcus* strains (representing 25 species) were tested. All species were either phylogenetically related to *S. aureus* or commonly found associated with the human body. All strains were tested at a concentration ranging from 0.7X10<sup>8</sup> to 3X10<sup>8</sup> CFU/mL or 0.7X10<sup>8</sup> to 3X10<sup>8</sup> target copies/mL. None of these samples tested positive, hence the analytical specificity was 100%.

## **Analytical Sensitivity**

The analytical sensitivity (limit of detection or LOD) of BD GeneOhm™ StaphSR was determined with 7 strains of MRSA that represent 6 MREJ genotypes (i, ii, iii, iv, v, and vii) and 4 SCCmec types (I, II, III, IV) and with 2 strains of MSSA. The LOD was defined as the lowest concentration, in DNA copy number or colony forming units (CFU) per reaction, at which five replicates out of five were found positive.

The LOD of the BD GeneOhm™ StaphSR Assay, for both MRSA and *S. aureus* (SA), is 15 DNA copies per reaction or 10 CFU per reaction. However, due to the enrichment process involved in blood cultures, all blood culture specimens contain very high bacterial loads resulting in DNA copy numbers per reaction well above the LOD of the assay.

In addition to the LOD determination, 100 MRSA strains (representing 29 countries) and 199 MSSA strains (representing 18 countries) from well characterized clinical isolates or public collections were evaluated using the BD GeneOhm™ StaphSR assay. MRSA strains were tested at a concentration of approx. 10<sup>2</sup> CFU/μL and MSSA strains were tested at a concentration of approx. 10<sup>5</sup> CFU/μL; MSSA strains were tested at the higher concentration to detect any cross-hybridization that could potentially lead to false positive results. The assay correctly identified all 100 MRSA strains. For the 199 MSSA strains, 187 were correctly identified as SA; the remaining 12 strains were further evaluated and were determined to be revertants; these strains tested positive for MRSA, as expected.

## **Reproducibility**

The reproducibility panel consisted of 12 simulated specimens containing 50 μL of a positive blood culture inoculated with either *Staphylococcus epidermidis*, MRSA or *S. aureus*; additionally, two controls (positive and negative) were included. The specimens were tested in triplicate on three different days at three different sites (12 specimens plus two controls tested X 3 X 3 days X 3 sites). This was repeated with three lots of reagents.

Two negative specimens were inoculated with *Staphylococcus epidermidis* and cultured until growth was detected. Ten positive specimens (5 MRSA and 5 *S. aureus*) were prepared from individual blood culture bottles that were inoculated with MRSA and *S. aureus*, respectively. Four serial dilutions were prepared in order to obtain different concentrations of bacteria for each specimen (Table 6).

Due to the enrichment process involved in blood cultures, all blood culture specimens contain very high bacterial loads compared to the LOD of the assay. Therefore the panel was not created based on the LOD but simply by dilution of a declared positive bottle. Thus, the panel aims to mimic potential calibration issues with automated blood culture systems, which may lead to early positivity determinations (i.e. lower bacterial loads than expected).

**Table 6. Cumulative data of reproducibility study**

Specimen ID	Dilution	Lot 1	Lot 2	Lot 3	Total agreement	Total % agreement
Negative	N/A	27/27	27/27	27/27	81/81	100%
Negative	N/A	27/27	27/27	27/27	81/81	100%
MRSA	1.0	27/27	27/27	27/27	81/81	100%
MRSA	1.0 E-01	27/27	27/27	27/27	81/81	100%
MRSA	5.0E-02	27/27	27/27	27/27	81/81	100%
MRSA	1.0 E-02	27/27	27/27	27/27	81/81	100%
MRSA	1.0 E-03	27/27	27/27	26/26	80/80	100%
SA*	1.0	27/27	27/27	27/27	81/81	100%
SA*	1.0 E-01	27/27	27/27	27/27	81/81	100%
SA*	5.0E-02	27/27	27/27	27/27	81/81	100%
SA*	1.0 E-02	27/27	27/27	27/27	81/81	100%
SA*	1.0 E-03	27/27	27/27	27/27	81/81	100%
Positive control	N/A	26/27	27/27	26/27	79/81	97.5%
Negative control	N/A	27/27	27/27	27/27	81/81	100%
Total agreement		377/378	378/378	376/377	1131/1133	99.8%
% of agreement		99.7%	100%	99.7%	99.8%	

\* SA in this panel represents a methicillin susceptible strain

## References

- <sup>1</sup> Centers for Disease Control and Prevention. Methicillin-resistant *Staphylococcus aureus* skin or soft tissue infection in a state prison. Mississippi, 2000. MMWR 2001; 50:919-922.
- <sup>2</sup> National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 to June 2004, issued October 2004. Am J Infect Control. 2004; 32:470-485.
- <sup>3</sup> Collignon, P. *et al.*, *Staphylococcus aureus* bacteremia, Australia. Emerg. Infect. Dis., 2005; 11, (4): 554-561.
- <sup>4</sup> Wyllie, D., *et al.*, Mortality after *Staphylococcus aureus* bacteraemia in two hospitals in Oxfordshire, 1997-2003: cohort study. BMJ, 2006; doi:10.1136/bmj.38834.421713.2F.
- <sup>5</sup> *Staphylococcus aureus* bacteremia: England, Wales and Northern Ireland: January to December 2003. CDR Weekly, 2004; 14 (16): 1-5.
- <sup>6</sup> Cosgrove, S., *et al.*, Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis., 2003, 36(1):53-9.
- <sup>7</sup> Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories. Richmond JY and McKinney RW (eds) (1999). HHS Publication number (CDC) 93-8395.
- <sup>8</sup> Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (current edition).
- <sup>9</sup> Clinical and Laboratory Standards Institute. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline, Document C24 (current edition).











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	Temperature limitation
	Protect from light and moisture
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	Consult instructions for use
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