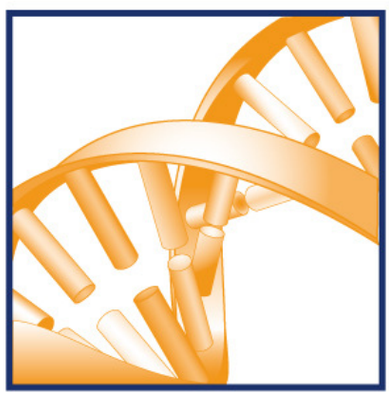
BD GeneOhm™ MRSA ACP Assay
Amplification Kit



IVD

REF 441637 48 Tests
REF 441639 200 Tests

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ENGLISH

Intended Use

The BD GeneOhm™ MRSA ACP Assay is a qualitative *in vitro* diagnostic test for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The test utilizes polymerase chain reaction (PCR) for the amplification of MRSA DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD GeneOhm™ MRSA ACP Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. It is not intended to diagnose MRSA infections nor to guide or monitor treatment for MRSA infections. Concomitant cultures are necessary only to recover organisms for epidemiological typing or for further susceptibility testing.

Summary and Explanation

MRSA is a major cause of nosocomial infections. Most transmissions occur through the contaminated hands of a person carrying MRSA. MRSA causes infections with clinical manifestations ranging from pustules to sepsis and death.[1] However, it may also be found in the nose or on the skin of healthy individuals (asymptomatic carriers). Treatment of MRSA infections has become a real challenge due to its broad resistance to antimicrobial agents. Methicillin-resistant strains of *S. aureus* are frequently encountered in healthcare settings, and represent over 50% of isolates from hospital acquired *S. aureus* in some North American hospitals[2]. MRSA prevalence continues to increase within U.S. hospitals and in the community.[2,3] Risk factors for acquisition of MRSA within healthcare facilities include prolonged hospital stay, proximity to patients infected or colonized with MRSA, colonization with other resistant organisms such as vancomycin-resistant enterococci (VRE) and *C. difficile*, exposure to multiple and/or prolonged antibiotic treatment, exposure to high MRSA prevalence areas within the healthcare facility, and prior MRSA infection or carriage. Early identification of patients with MRSA carriage is an important part of an effective infection prevention program for MRSA. Culture-based detection of MRSA requires isolation of pure colonies followed by either oxacillin or cefoxitin susceptibility testing, detection of the *mecA* gene or detection of the penicillin binding protein (PBP 2a) encoded by the *mecA* gene. The culture based process takes a minimum of 24 hours with a median time to result closer to 48 hours in order to identify MRSA. With the rapidity at which MRSA infections can spread, especially in healthcare settings where carriers are common, the capability of determining MRSA nasal carriage on the day of admission represents a definite advantage for infection prevention programs.

Principle of the Procedure

A nasal specimen is collected and transported to the laboratory using a recommended swab (refer to “Materials Required But Not Provided” section). The lysis of bacterial cells in nasal swab specimens is performed using the BD GeneOhm™ MRSA ACP Lysis kit. An aliquot of the lysate is added to prepared PCR reagents which contain MRSA-specific primers that will amplify in the presence of genetic target. The assay also includes an Internal Control (IC) to monitor for the presence of inhibitors in the PCR reaction and to confirm the integrity of assay reagents. Controls and specimen lysates are added to disposable reaction tubes and placed in the SmartCycler® II instrument. The amplification, detection and results interpretation are automatically performed by the SmartCycler® II software. The BD GeneOhm™ MRSA ACP Assay procedure can be performed within 2 hours, depending on the number of specimens processed. To recover MRSA for epidemiological typing or for further antibiotic susceptibility testing, appropriate culture media can be inoculated during or up to 24 hours after specimen preparation.

The primers and probes in the BD GeneOhm™ MRSA ACP Assay detect a proprietary sequence inserted into the *S. aureus* chromosome indicating the presence of MRSA DNA. Amplification of IC and MRSA DNA are detected using specific hybridization probes that bind to a specific sequence of the amplified target. Differentiation of MRSA DNA and IC is done using molecular beacons which contain different fluorometric properties. The beacon-target hybrid fluoresces at a different wavelength for MRSA and IC and the emitted light from this reaction is measured by the SmartCycler® II instrument. MRSA or IC specific amplicons are detected simultaneously in two different fluorescence channels on the SmartCycler and can therefore be differentiated. The operation of the SmartCycler® II instrument is based on the proprietary microprocessor-controlled I-CORE® (Intelligent Cooling/Heating Optical Reaction) module[4].

Reagents

BD GeneOhm™ MRSA ACP Assay	**48 Tests**	**200 Tests**
Master Mix	8 tubes	30 tubes
< 0.0005% DNA polymerase complex		
< 0.001% Internal Control: non-infectious DNA containing MRSA-primer binding sequences and a unique sequence for probe hybridization		
< 0.06% primers		
< 0.02% Molecular probes		
< 1% Nucleotide mix (dATP, dCTP, dGTP, dTTP)		
Bovine serum albumin		
Carbohydrate		
$MgCl_2$		
< 0.001% non-infectious *Staphylococcus epidermidis* genomic DNA (ATCC 14990)		
Control DNA	8 tubes	30 tubes
Tris-EDTA buffer		
Carbohydrate		
< 0.001% non-infectious genomic MRSA DNA (ATCC 43300)		
Diluent	8 X 700 µL	30 X 700 µL
Tris-HCl buffer		
$MgCl_2$		
$(NH_4)_2SO_4$		
KCl		
Tween-20		

Precautions

- For *in vitro* diagnostic use.
- Do not use the kit if the outer carton safety seal is broken.
- Do not use reagents if the protective pouches are open or torn upon arrival.
- Close protective pouches of Master Mix and Control DNA promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not remove desiccant from Master Mix and Control DNA pouches.
- Do not use reagents if desiccant is not present or broken inside 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 dates.
- 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 MRSA amplicons, do not open the reaction tubes post-amplification.
- Use a pipettor tip with a diameter small enough to reach the liquid at the bottom of the specimen or reagent tube
- Performing the assay outside of the recommended time ranges may produce invalid results. Assays not performed 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.
- If other open-tube PCR tests are performed by the laboratory, separated and segregated work 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 moving 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[5] and in the CLSI Document M29[6].
- 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

- Master Mix
- Control DNA
- Diluent
- SmartCycler® reaction tubes, 25 µL capacity

Storage, Handling and Stability

Note: Storage conditions must follow the specifications written on each pouch. Preparation of Master Mix and Positive and Negative Controls must be performed in an environment where temperatures do not exceed 25°C.

Kit components		**Master Mix (white strip label), Control DNA (red strip label) and Diluent (black strip label) Storage Conditions**
Sealed pouch	**Temperature**	2-25˚C
	Stability	Expiration date
Opened pouch	**Temperature**[1]	2-8˚C
	Stability[2]	1 month

[1] Once the original seal on the pouch is broken, remove excess air and carefully close the pouch with the zip seal after each use and store at appropriate temperature..
[2] Provided bag is properly closed with the zip seal after each use.

Kit Components outside of their protective pouch			**Master Mix (white strip label) and Control DNA (red strip labels)**
Tubes containing unreconstituted reagents		**Temperature**	15-25˚C
		Stability	2 hours
Tubes containing reconstituted reagents[1]	**Original container**	**Temperature**	2-25 ˚C
		Stability[2]	3 hours
	SmartCycler® tube	**Temperature**	2-25˚C
		Stability[2]	Procedure steps 5 - 10: 30 minutes Procedure steps 11- 15: 30 minutes

[1] Discard unused tubes after expiration of indicated stability.
[2] If kept on ice or in cooling block.

Materials Required But Not Provided

- BD GeneOhm™ MRSA ACP Lysis Kit (BD catalog no. 441638)
- Vortex Genie 2 (VWR catalog no. 58815-234) with 1.5 mL microtube holder or equivalent; for processing multiple samples, adaptor designed for multiple tubes can be used (VWR catalog no. 58816-146)
- Calibrated Micropipettors (accurate range between 1-10 µL, 10-100 µL and 100-1000 µL)

- Sterile DNase-free filter-blocked or positive displacement micropipettor tips [1-10 µL (46 mm), 10-100 µL and 100-1000 µL]
- Ice or cooling block for 1.5 mL and SmartCycler® tubes
- Microcentrifuge for SmartCycler® tubes
- SmartCycler® II starter system with Dx Software (processing/cooling block, user manual, accessory kit, and computer)
- Cap removal tool (e.g. MATRIX catalog no. 4469) (optional)
- Disposable gloves, powderless
- Stopwatch or timer
- BD GeneOhm™ MRSA ACP Assay Definition CD (contact BD Customer Service for installation support)

Instructions for Use

Preparation

Refer to the **BD GeneOhm™ MRSA ACP Lysis Kit** package insert for instructions on Bulk Lysis Reagent Preparation, Specimen Collection, Specimen Preparation, Specimen Lysis, and Culturing of Clinical Specimens.

Two (2) PCR Controls (one (1) Positive and one (1) Negative) **must be included in each BD GeneOhm™ MRSA ACP Assay run.** Refer to the BD GeneOhm™ MRSA ACP Lysis Kit package insert for instructions on preparing PCR controls.

BD GeneOhm™ MRSA ACP Assay

Note: One (1) reconstituted Master Mix tube (white label) will yield up to eight (8) reactions. One (1) SmartCycler® tube is required for every specimen. Two (2) additional SmartCycler® tubes are required for PCR controls. Remove the required number of tubes from their protective pouch, remove the excess air and close the pouch promptly with the zip seal. Ensure the protective pouch is properly sealed after closing. The maximum number of SmartCycler® tubes per run is based on the number of available I-CORE® modules on the SmartCycler® II instrument(s).

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 cap of the Master Mix tube. Do not insert the tip too deeply into the cap. Dispense the diluent into the tube. Discard remaining unused diluent.
3. **Vortex the tube(s) for 5-10 seconds.**

   Place the reconstituted Master Mix tube on ice or on a cooling block designed for 1.5 mL tubes until ready for processing.
4. **Place the required number of SmartCycler® tubes on the SmartCycler® cooling block.**

   Label each SmartCycler® tube with the appropriate identification. Avoid touching the optical detection windows at the bottom edges of the tube and the lower diamond-shaped area.

The Following Steps (5 to 10) MUST be performed WITHIN 30 MINUTES

NOTE: To ensure timely completion of steps 5-10, it is recommended to centrifuge SmartCycler® tubes in sets of 8 once samples have been prepared.

5. **Add 25 µL of reconstituted Master Mix to each SmartCycler® tube.**

   Carefully remove the septum cap before pipetting the reagent. Dispense reconstituted Master Mix reagent into the reservoir (upper part) of each SmartCycler® tube.

NOTE: If septum cap was used to seal lysis tubes, then 46 mm pipette tips are required for Steps 6 through 8.

6. **Add 3 µL of lysed specimen to each corresponding labeled SmartCycler® tube containing Master Mix reagent. Close the SmartCycler® tube tightly after addition of each specimen lysate.**

   After addition of the specimen, gently prime the pipet tip 2-3 times in the SmartCycler® tube reservoir to ensure complete volume of specimen is dispensed. **Use a new micropipettor tip for each specimen.**

7. **Add 3uL of Positive control to the appropriately labeled SmartCycler® tube. Close the SmartCycler® tube tightly.**
8. **Add 3uL of Negative control to the appropriately labeled SmartCycler® tube. Close the SmartCycler® tube tightly.**
9. **Place the lysis tubes, containing the unused portion of lysates, at -20 ˚C for further testing (if needed).**
10. **Centrifuge all reaction tubes for 5-10 seconds.**

    Use a microcentrifuge compatible with SmartCycler® tubes.

The Following Steps (11 to 15) MUST be performed WITHIN 30 MINUTES:

11. **Maintain the tubes at 2-8°C on the SmartCycler® cooling block before loading on the instrument.**
12. **Create a run with the "BD GeneOhm MRSA ACP" protocol.**

    Enter the number of samples to be run. Refer to the SmartCycler® Dx Software Operator Manual if needed.

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

    Place the assay Positive and Negative Controls in their appropriate positions as assigned by the Smartcycler® Dx software.

14. **Press down on the top of each tube to ensure tubes are firmly in place.**
15. **Start the run. Enter each specimen identification either manually or using a barcode reader.**

Repeat Testing Procedure for PCR Control Failure and Specimen Processing Control (SPC) Failure (When SPCs are Performed)

Note: Original Sample Buffer tubes (containing eluted specimens and controls) stored at 2-8°C for up to 72 hours may be used for repeat testing (refer to the "Storage Handling and Stability" section of the BD GeneOhm™ MRSA ACP Lysis Kit package insert).

1. **Place all tubes (specimen and controls) used for the repeat run on a cooling block at 2-8°C.**

   If SPCs have been prepared along with the specimens requiring repeat testing, include SPC Sample Buffer tubes on the cooling block at 2-8°C.

2. **Follow steps 4 to 10 of the "Specimen Lysis" section in the BD GeneOhm™ MRSA ACP Lysis Kit package insert.**
3. **Follow steps 1 to 15 of the "BD GeneOhm™ MRSA ACP Assay" section above.**

Repeat Testing Procedure for Unresolved or Not Determined Results

Note: Specimens and controls should be repeated from the frozen specimen/control lysate tubes, stored no longer than 8 days at -20°C.

Within a run that includes new samples:

1. **Prepare new specimens and controls according to instructions provided in the BD GeneOhm™ MRSA ACP Lysis Kit package insert.**
2. **Thaw the frozen specimen lysates to be retested.**
3. **Place the lysates on a cooling block at 2-8°C. Vortex the lysates for 5-10 seconds and centrifuge for 5-10 seconds (quick spin) to ensure that the liquid is located at the bottom of the tubes.**
4. **Repeat steps 1 to 15 of the "BD GeneOhm™ MRSA ACP Assay" section above for controls, thawed specimens and new specimens.**

Within a run that includes only repeat samples (from one or more worklists):

1. **Thaw the frozen specimen and control lysates to be retested.**
2. **Place the lysates on a cooling block at 2-8°C. Vortex the lysates for 5-10 seconds and centrifuge for 5-10 seconds (quick spin) to ensure that the liquid is located at the bottom of the tubes.**
3. **Repeat steps 1 to 15 of the "BD GeneOhm™ MRSA ACP Assay" section above.**

Quality Control

Positive and Negative PCR Controls

Quality control procedures are designed to monitor assay performance. The Positive Control is intended to monitor for substantial reagent failure. The Negative Control is used to detect reagent or environmental contamination (or carry-over) by either MRSA DNA or MRSA amplicons. Positive and Negative Controls are assay controls (run controls). Finally, an Internal Control (IC) is incorporated within each reaction mixture to monitor reagent integrity and PCR inhibition within each specimen.

One (1) Positive Control and one (1) Negative Control must be included in each BD GeneOhm™ MRSA ACP Assay run. These controls must be incubated and inactivated with the lysis reagent as described in the BD GeneOhm™ MRSA ACP Lysis Kit package insert. The PCR software automatically assigns the position of the controls on the instrument (refer to the SmartCycler® Dx Software Operator Manual). All Positive and Negative Controls should yield valid results. For MRSA DNA negative specimens, Internal Controls should yield valid results (refer to "Interpretation of Results" section).

Specimen Processing Controls (SPC)

Specimen Processing Controls (SPCs) are recommended in order to provide assurance that significant cross contamination does not occur and to monitor for substantial reagent or methodology failure during the assay process. Additional control strains should be tested as SPCs according to guidelines or requirements of local, state and/or federal regulations or accreditation organizations. A reference MRSA strain (e.g. ATCC 43300) or a well characterized MRSA clinical isolate may be used as a positive SPC; MRSA MREJ type iii and vii strains, if available, may be used as additional positive SPC, to monitor assay probes and primers not directly controlled in the assay. A strain of methicillin susceptible *Staphylococcus aureus* (e.g. ATCC 25923) or any other non-*aureus Staphylococci* (e.g. *Staphylococcus epidermidis* ATCC 14990) may be used as a negative SPC.

SPCs should yield valid results when performed. In the event of an incorrect SPC result, it is highly recommended that the specimens within the same sample processing run as the SPCs, be retested, in accordance with the procedure outlined in the "Repeat Testing Procedure for PCR Control Failure and Specimen Processing Control (SPC) Failure (When SPCs are Performed)" section, before reporting results.

For general QC guidance, the user may wish to refer to CLSI C24[3] and MM3.[4]

Interpretation of Results

The decision algorithm for the BD GeneOhm™ MRSA ACP Assay is embedded in the SmartCycler® software. The interpretation of assay results is performed according to the following criteria:

Assay result reported	IC result reported	Interpretation of result[1]
NEG	PASS	No MRSA DNA detected
POS	NA	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[2])

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

[1] BD GeneOhm™ MRSA ACP Assay results may be used to guide isolation and level of precautions in accordance with institutional programs and practices.

[2] Refer to the SmartCycler® Dx Software Operator Manual for interpretation of warning and error codes.

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

Invalid Run

An Invalid Positive or Negative PCR Control Invalidates the Assay Run. In such cases, assay results obtained for that run are invalid and must not be reported. Invalid assay run or instrument error codes or warnings are flagged on reports. Before reporting MRSA results, always verify that the assay run is valid. Please refer to the "Repeat Testing Procedure for PCR Control Failure and Specimen Processing Control (SPC) Failure (When SPCs are Performed)" section.

Unresolved Specimen

Repeat testing with the corresponding frozen lysates for specimen(s) and controls. The freeze-thaw cycle has been shown to reduce PCR inhibitory substances in the specimen lysate. Please refer to the "Repeat Testing Procedure for Unresolved or Not Determined" section.

Specimen Not Determined Due to I-CORE® Module Failure

Repeat testing with the corresponding frozen lysates for specimen(s) and controls. Please refer to the "Repeat Testing Procedure for Unresolved or Not Determined" section. For the interpretation of warning or error code messages, refer to the SmartCycler® Dx Software Operator Manual.

Limitations of the Procedure

- This product should only be used with nasal swab specimens lysed using the BD GeneOhm™ MRSA ACP Lysis Kit.
- This product should only be used with the SmartCycler® II instrument.
- Negative test results may also occur from improper specimen collection, handling or storage, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the package insert instructions and in the Operator Manual(s) are necessary to avoid erroneous results.
- 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.
- Screening determines the colonization status at a given time, which could vary depending upon patient treatment (e.g. decolonization regime), patient status (e.g. not actively shedding MRSA) or exposure to high risk environments (e.g. contact with MRSA carrier, prolonged hospitalization). Colonization status should be monitored according to institutional policies.
- Results of the BD GeneOhm™ MRSA ACP Assay should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions. The test is not intended to identify patients with staphylococcal infection. Results should not be used to guide or monitor treatment for MRSA infections.
- A BD GeneOhm™ MRSA ACP positive result does not necessarily indicate treatment eradication failure since DNA presence may persist. A negative result following a previously positive test result may indicate treatment eradication success or may occur due to intermittent shedding.
- A positive test result does not necessarily indicate the presence of viable organisms. However a positive result is indicative for the presence of MRSA DNA since the BD GeneOhm™ MRSA ACP Assay simultaneously detects the SCC*mec* cassette (carrying the *mecA* gene) and a *S. aureus* specific sequence located within the *orfX* gene.
- Twenty (20) MREJ genotypes (MREJ genotypes i to xx) have been described in the literature based on sequence analyses of the SCC*mec*/*orfX* junction of different clinical isolates of MRSA. The MREJ genotype does not correlate with the SCC*mec* type, i.e., different MREJ genotypes can be associated with the each of the eight (8) known SCC*mec* types. The BD GeneOhm™ MRSA ACP Assay is designed to detect MREJ genotypes i, ii, iii, iv, v and vii only; these six (6) MREJ genotypes account for more than 98% of worldwide strains tested by BD Diagnostics to date. The BD GeneOhm™ MRSA ACP Assay may not detect other MREJ genotypes resulting in false negative results.
- The BD GeneOhm™ MRSA ACP Assay does not detect the *mecA* gene directly nor the penicillin binding protein (PBP 2a) encoded by this gene. A false positive MRSA result may occur if an "empty cassette" *S. aureus* variant is present.
- The BD GeneOhm™ MRSA ACP Assay may cross-react with certain strains of methicillin-sensitive *Staphylococcus aureus* (MSSA) when they are present at extremely high concentrations. The BD GeneOhm™ MRSA ACP Assay generated 5 false positive results during an analytical specificity

study testing a total of 111 well characterized MSSA strains at approximately 3 x 10^5 genome copies/PCR reaction (~ 7 x 10^7 CFU/swab).

- An excess amount of blood in a specimen may inhibit the BD GeneOhm™ MRSA ACP Assay. Rhinaris® and Secaris® at high concentrations may cause slight inhibition in the BD GeneOhm™ MRSA ACP Assay (refer to "Interfering Substances" section for further details).
- As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the LoD of the assay may be detected, but results may not be reproducible (refer to "Reproducibility" section for further details).
- False negative results may occur due to loss of nucleic acid from inadequate collection, transport or storage of specimens, or due to inadequate bacterial cell lysis. The Internal Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Internal Control does not indicate if nucleic acid has been lost due to inadequate collection, transport or storage of specimens, or if bacterial cells have been adequately lysed.
- BD GeneOhm™ MRSA ACP Assay results may sometimes be unresolved or invalid due to an invalid control, and require retesting that can lead to a delay in obtaining final results.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MRSA variants resulting in a false negative result with the BD GeneOhm™ MRSA ACP Assay.
- Preparation of Master Mix and Positive and Negative Controls must be performed in an environment that does not exceed 25°C.
- As with all *in vitro* diagnostic tests, positive and negative predictive values are highly dependent on prevalence. BD GeneOhm™ MRSA ACP Assay performance may vary depending on the prevalence and population tested.

Expected Values

In the BD GeneOhm™ MRSA ACP Assay clinical study, a total of 1228 specimens were tested from three geographically diverse U.S. clinical sites. The study population was grouped into subjects in Intensive Care, Long Term Care Facility, and Medical Services. The number and percentage of positive and negative cases as determined by the comparative culture reference method are calculated and presented in the table below:

		MRSA By Culture		
Group	**Total N**	**Number Positive**	**Number Negative**	**Observed Prevalence**
Intensive Care	137	10	127	7.3%
Long Term Care Facility	263	58	205	22.1%
Medical Services[1]	828	121	707	14.6%
Total	1228	189	1039	15.4%

[1] The Medical Services category includes specimens identified as Medical, OB/GYN, Pediatric, Surgical and Other.

Performance Characteristics

Clinical Performance

Clinical performance characteristics of the BD GeneOhm™ MRSA ACP Assay were determined in a multi-site prospective investigational study. Three (3) investigational centers participated in the study. To be enrolled in the study, patients had to be eligible for MRSA testing according to institutional policies. Eligibility requirements for targeted screening as per clinical site policies included, but were not limited to: all patients admitted into the particular healthcare system; patients admitted to the Intensive Care Unit; patients transferred to the Intensive Care Unit; pre-elective surgery patients; and patients being admitted from long term care facilities. Patients previously enrolled in the study were denied repeat entry.

The Comparative Reference Method consisted of an initial analysis on a selective chromogenic media followed by subculture on Blood Agar (BA) of presumptive *S. aureus* colonies. Identification was confirmed with an agglutination test, while methicillin-resistance was confirmed by cefoxitin disk diffusion susceptibility testing. Enrichment in Trypticase Soy Broth (TSB) was completed in the event that methicillin-resistant *S. aureus* was not confirmed by the initial method. The TSB was used to inoculate additional chromogenic media and BA plates, and MRSA confirmation was performed as described above.

A total of 1228 nasal swab specimens were tested with both the Comparative Reference Method and the BD GeneOhm™ MRSA ACP Assay. There were 1216 reportable results (Table 1); twelve (12) nasal swab specimens were excluded from performance analysis due to non-compliance with the clinical study protocol control strategy. In comparison to the Comparative Reference Method, the BD GeneOhm™ MRSA ACP Assay identified 92.0% of the MRSA positive specimens and 94.6% of the negative specimens (Table 2). For the population tested, this resulted in a Negative Predictive Value (NPV) of 98.5% and a Positive Predictive Value (PPV) of 75.4%.

Out of 1216 nasal swab specimens tested with the BD GeneOhm™ MRSA ACP Assay, 12 (1.0%) were initially reported as unresolved (Table 3). Upon repeat testing from the frozen lysates, all 12 had reportable results. Three (3) runs were reported invalid due to Run Control failure (4.4%) (Table 4). The runs were reported valid upon repeat testing of the specimen lysates.

Table 1: Results Obtained with the BD GeneOhm™ MRSA ACP Assay in Comparison with the Reference Method

		Comparative Reference Method +	Comparative Reference Method -	
BD GeneOhm™ MRSA ACP Assay	**+**	172	56	228
	-	15	973	988
		187	1029	1216

Table 2: Performance Obtained using the BD GeneOhm™ MRSA ACP Assay in Comparison with the Reference Method

Clinical Sites	Prevalence	Sensitivity with 95% CI*	Specificity with 95% CI*
Site 1	11.6% (67/579)	94.0% (85.4%, 98.3%)	96.7% (94.7%, 98.1%)
Site 2	17.5% (56/320)	88.9% (77.4%, 95.8%)	89.8% (85.4%, 93.2%)
Site 3	20.1% (66/329)	92.4% (83.2%, 97.5%)	95.1% (91.7%, 97.3%)
Overall	15.4% (189/1228)	92.0% (87.1%, 95.4%)	94.6% (93%, 95.9%)

* CI: Confidence Intervals

Table 3: Unresolved Rates

Clinical Sites	Initial unresolved rate with 95% CI*		Unresolved rate after repeat with 95% CI*	
Site 1	1.0% (6/579)	(0.4% - 2.2%)	0.0% (0/579)	(0.0% - 0.6%)
Site 2	0.3% (1/308)	(0.0% - 1.8%)	0.0% (0/308)	(0.0% - 1.2%)
Site 3	1.5% (5/329)	(0.5% - 3.5%)	0.0% (0/329)	(0.0% - 1.1%)
Overall	1.0% (12/1216)	(0.5% - 1.7%)	0.0% (0/1216)	(0.0% - 0.3%)

* CI: Confidence Intervals

Table 4: Overall Invalid Run Rates

Site	Invalid Run Rates with 95% CI*	
Site 1	0.0% (0/25)	(0.0% - 13.7%)
Site 2	4.5% (1/22)	(0.1% - 22.8%)
Site 3	9.5% (2/21)	(1.2% - 30.4%)
Overall	4.4% (3/68)	(0.9% - 12.4%)

* CI: Confidence Intervals

Analytical Sensitivity

Limit of Detection (LoD) Determination Using Genomic DNA

The analytical sensitivity (limits of detection or LoDs) of the BD GeneOhm™ MRSA ACP Assay testing genomic DNA were determined using decreasing amount of quantified (copies/PCR reaction) genomic DNA from cultures of 6 MRSA strains that represent 6 MREJ genotypes (i, ii, iii, iv, v, and vii) and 4 SCC*mec* types (I, II, III, IV). Each MRSA strain was tested in replicates of 48 per concentration by 2 different operators using 3 different lots of lyophilized BD GeneOhm™ MRSA ACP Assay Master Mixes. Analytical sensitivity (LoD), defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranged from 2.5 to 5 copies/PCR reaction, with an average value of 5 DNA copies/PCR reaction.

MRSA Strain	MREJ Genotype	SCCmec Type	LoD Concentration
1	type i	I	5 copies/PCR reaction
2	type ii	II	5 copies/PCR reaction
3	type iii	III	5 copies/PCR reaction
4	type iv	III	5 copies/PCR reaction
5	type v	IV	2.5 copies/PCR reaction
6	type vii	II	5 copies/PCR reaction

Limit of Detection (LoD) Determination Using Viable Bacteria with Clinical Nasal Matrix

The analytical sensitivity (limits of detection or LoDs) of the BD GeneOhm™ MRSA ACP Assay testing viable MRSA strains with clinical nasal matrix were determined using simulated positive swabs that were prepared by soaking swabs in a wide range of MRSA bacterial suspensions prepared and quantified from cultures of 6 MRSA strains that represent 6 MREJ genotypes (i, ii, iii, iv, v, and vii) and 4 SCC*mec* types (I, II, III, IV), and then discharged/eluted in pooled negative clinical nasal matrix. Each MRSA strain was tested in replicates of 24 per concentration by 2 different operators using 3 different lots of lyophilized BD GeneOhm™ MRSA ACP Assay Master Mixes. Analytical sensitivity (LoD), defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranged from 130 to 576 CFU/swab, with an average value of 300 CFU/swab.

MRSA Strain	MREJ Genotype	SCC*mec* Type	LoD Concentration
1	type i	I	207 CFU/swab
2	type ii	II	576 CFU/swab
3	type iii	III	256 CFU/swab
4	type iv	III	245 CFU/swab
5	type v	IV	130 CFU/swab
6	type vii	II	386 CFU/swab

Analytical Inclusivity

Analytical ubiquity of the BD GeneOhm™ MRSA ACP Assay was evaluated in the analytical inclusivity study. A variety of *Staphylococcus aureus* strains were included in the study taking into account geographic origin, MREJ genotype, SCC*mec* type, pulse field gel electrophoresis (PFGE) type, temporal diversity and susceptibility pattern. One-hundred-forty (140) strains from 31 countries were tested in this analytical inclusivity study, including 52 from public collections and 88 from well-characterized clinical isolates, including Vancomycin-resistant *Staphylococcus aureus* (VRSA) and Vancomycin-intermediate *Staphylococcus aureus* (VISA) strains.

When tested at the relevant clinical load (roughly 100 genome copies/µL), 99% of all the strains were detected. The BD GeneOhm™MRSA ACP Assay detected all of the MREJ wild types i, ii, iii, iv, v and vii tested, as well as MREJ mutant types ii mut16, ii mut25 and iii mut25. The BD GeneOhm™ MRSA ACP Assay detected MRSA SCC*mec* types I, II, III, IV, V and VI, as well as MRSA PFGE types USA 100 to 800, 1000 and 1100. All *Staphylococcus aureus* strains displaying additional resistance to vancomycin (VRSA and VISA) were also detected. When the same strains were tested in triplicate at concentrations < 3X LoD, 83% were detected in all three replicates and 99% were detected in at least one out of three replicates.

Evaluation of a Well Characterized Challenge Strain Panel

An additional analytical study was carried out to evaluate the analytical performance of the BD GeneOhm™ MRSA ACP Assay by testing a well characterized challenge strain panel containing MRSA strains with high and low oxacillin minimum inhibitory concentrations (MICs), including PFGE types USA 100, 300, and 400 (with emphasis on USA 300), BORSA (borderline oxacillin-resistant *S. aureus* strains are *mec*A negative, but exhibit oxacillin resistance by a mechanism not completely understood), methiciilin-sensitive *S. aureus* (MSSA), and methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains. The challenge strain panel used in this study was composed of 15 MRSA, 4 BORSA, 1 MRSE and 5 MSSA strains. All MRSA strains tested belong to MREJ type ii, with the exception of one MRSA strain, which belonged to MREJ type iii. These strains have previously been shown to display a broad range of oxacillin and cefoxitin MICs. All these strains were tested with FDA-cleared broth dilution susceptibility tests for determination of the MIC values.

All the MRSA strains tested (including PFGE types USA 100, 300 and 400) exhibited positive results when tested at 2-3X LoD concentration. All BORSA, MSSA and MRSE strains tested exhibited negative results when tested at high concentrations.

Analytical Specificity

Forty-one (41) out of 42 strains of various non-staphylococcal species tested at a concentration corresponding to ~ 3 x 10^5 copies/PCR reaction (~ 7 x 10^7 CFU/swab) produced negative results with the BD GeneOhm™ MRSA ACP Assay. One (1) strain was reported as positive by the BD GeneOhm™ MRSA ACP Assay. Investigation demonstrated that the positive result obtained was due to MRSA contamination.

Nineteen (19) Methicillin susceptible Coagulase Negative *Staphylococci* (MSCNS) strains, 15 Methicillin resistant Coagulase Negative *Staphylococci* (MRCNS) strains and 2 Coagulase Negative *Staphylococci* (CNS) strains tested with the BD GeneOhm™ MRSA ACP assay produced negative results. The specificity with Coagulase Negative *Staphylococci* was 100%.

106 out of 111 MSSA strains tested at extremely high concentrations, ~ $3x10^5$ copies/PCR reaction (~ 7 x 10^7 CFU/swab), produced negative results with the BD GeneOhm™ MRSA ACP assay; the remaining 5 MSSA strains produced negative results at ~ $3x10^4$ copies/PCR reaction (~ 7 x 10^6 CFU/swab). Therefore, the specificity of the BD GeneOhm™ MRSA ACP Assay with MSSA was 100% (111/111) at ~ $3x10^4$ copies/PCR reaction (~ 7 x 10^6 CFU/swab), and 95.5% (106/111) at ~ $3x10^5$ copies/PCR reaction (~ 7 x 10^7 CFU/swab).

Interfering Substances

Eighteen (18) substances used in bacterial culture, transport media, as well as biological and chemical substances occasionally used in the nares or found in nasal swab specimens were evaluated for potential interference with the BD GeneOhm™ MRSA ACP Assay. MRSA positive specimens were tested at 3x the Limit of Detection (LOD) and at typical clinical concentration (Ct values in the FAM channel between 30.0 and 35.0) with the highest amount of each compound likely to be found at the sampling site or on the nasal swab specimens. Results demonstrated no reportable interference with any substance except for blood when present in excess. Rhinaris® and Secaris® at high concentrations showed slight inhibition in the BD GeneOhm™ MRSA ACP Assay, however, expected assay results were still obtained.

Endogenous and Commercial Exogenous Substances Tested with the BD GeneOhm™ MRSA ACP Assay

Substance	Result		Substance	Result
Dristan®	NI		Mannitol Salt Agar plate	NI
Drixoral®	NI		CHROMagar	NI
Flonase®	NI		Liquid Amies	NI
Nasonex®	NI		Liquid Stuart	NI
Otrivin®	NI		Gel Amies	NI
Petroleum jelly	NI		Blood	I
Rhinaris®	NI*		Nasal Secretion	NI
Secaris®	NI*		Cromolyn eye drops®	NI
Tryptic Soy Broth	NI		Saline	NI

NI: No reportable interference with the BD GeneOhm™ MRSA ACP Assay.
I: Detectable interference with the BD GeneOhm™ MRSA ACP Assay only if substance is present in excess.
* Rhinaris® and Secaris® at high concentrations showed slight inhibition in the BD GeneOhm™ MRSA ACP Assay, however, expected assay results were still obtained.

Reproducibility

The reproducibility panel consisted of 4 specimen categories near the LoD. Each tube contained simulated nasal flora (*Staphylococcus epidermidis* (ATCC 14990)). Two MRSA strains were tested in each of the 4 categories, as follows:

- Moderate Positive (MP): 2 - 5X LoD
- Low Positive (LP): 1- 2X LoD
- High Negative 1:10 (HN1:10): 10-fold dilution of 1X LoD
- High Negative 1:100 (HN1:100): 100-fold dilution of 1X LoD

A fifth category consisted of negative (Neg) specimens (simulated nasal flora and no MRSA).

Specimens in each category were tested in triplicate, on 5 distinct days, wherein each day 2 panels were tested by 2 technologists, at 3 clinical sites using 1 lot of reagents (Site-to-Site). One (1) of these clinical sites participated in an extended study where 2 additional lots of reagents were tested (Lot-to-Lot). Results are shown for each specimen category with the data from both MRSA strains pooled.

For Site-to-Site Reproducibility, the overall percent agreement was 100% for MP and Neg categories, 95.0% for LP, 88.3% and 47.2% negative agreement for HN1:100 and HN1:10 categories, respectively (Table 5).

For Lot-to-Lot Reproducibility, the overall percent agreement was 100% for MP and Neg categories, 98.3% for LP, 91.7% and 41.7% negative agreement for HN1:100 and HN1:10 categories, respectively (Table 6).

Cycle threshold (Ct), an internal criteria used to determine a final assay result, was selected as an additional means of assessing assay reproducibility. Overall mean Ct values with variance components (SD and %CV) are shown in Tables 5 and 6.

Table 5: Site-To-Site Reproducibility Study Results using One Lot

Category	SITE						Overall Percent Agreement		Ct Values[1]		
	Site 1		Site 2		Site 3						
	Percent Agreement		Percent Agreement		Percent Agreement				Overall Mean	SD	%CV
Neg	30/30	100%	30/30	100%	30/30	100%	90/90	100%	34.9	0.3	0.9
HN1:100[2]	49/60	81.7%	58/60	96.7%	52/60	86.7%	159/180	88.3%	40.8	1.6	3.8
HN1:10[2]	26/60	43.3%	27/60	45.0%	32/60	53.3%	85/180	47.2%	39.8	1.5	3.8
LP	59/60	98.3%	60/60	100%	52/60[3]	86.7%	171/180	95.0%	38.5	1.1	2.8
MP	60/60	100%	60/60	100%	60/60	100%	180/180	100%	36.8	1.0	2.6

[1] For the Neg category, CT values reported are for the internal control. For other categories, CT values reported are for the MRSA target.
[2] For the High Negative categories, the expected assay result was deemed to be negative. Therefore, percent agreement was calculated for negative results.
[3] Eight (8) LP specimens initially reported as negative were positive upon retesting from frozen lysates.

Table 6: Lot-To-Lot Reproducibility Study Results using Three Lots

Category	LOT						Overall Percent Agreement		Ct Values[1]		
	Lot 1		Lot 2		Lot 3						
	Percent Agreement		Percent Agreement		Percent Agreement				Overall Mean	SD	%CV
Neg	30/30	100%	30/30	100%	30/30	100%	90/90	100%	34.8	0.4	1.2
HN1:100[2]	58/60	96.7%	54/60	90.0%	53/60	88.3%	165/180	91.7%	39.9	1.1	2.7
HN1:10[2]	27/60	45.0%	28/60	46.7%	20/60	33.3%	75/180	41.7%	39.6	1.0	2.5
LP	60/60	100%	58/60	96.7%	59/60	98.3%	177/180	98.3%	38.2	0.9	2.4
MP	60/60	100%	60/60	100%	60/60	100%	180/180	100%	36.5	0.9	2.5

[1] For the Neg category, CT values reported are for the internal control. For other categories, CT values reported are for the MRSA target.
[2] For the High Negative categories, the expected assay result was deemed to be negative. Therefore, percent agreement was calculated for negative results.

Precision

Within-laboratory precision was evaluated for the BD GeneOhm™ MRSA ACP Assay at 1 site. The study was performed using the same specimen categories and calculations as above. Testing was performed in duplicate, over 12 days, with 2 runs per day, by 2 technologists. All samples and controls produced reportable results except for 1 HN1:100 sample and 2 HN1:10 samples which produced unresolved results. Repeat testing with the frozen lysates produced reportable results. Precision study results for Neg, LP and MP samples demonstrated 100% agreement. Precision study results for HN1:100 and HN1:10 demonstrated agreement of 95.8% and 41.7%, respectively.

Carry-Over Contamination

To evaluate the risk of carry-over while processing specimens with high MRSA bacterial load in the BD GeneOhm™ MRSA ACP Assay, an analytical study was carried out to evaluate the entire process from sample preparation (including utilization of septum and screw caps on lysis tubes) to PCR result. Two (2) MRSA strains (MREJ type ii and vii) were used as the high positive MRSA panel members (1.4 X 10^4 copies/PCR reaction and 5.1 X 10^4 copies/PCR reaction respectively). Negative members were prepared with Sample Buffer. Three (3) replicates of each high positive panel member (a total of 6 high positives) and 6 replicates of the negative panel member were tested by alternating negative and positive samples. Each run contained positive and negative controls prepared according to the package insert. All panel members and controls were processed following the BD GeneOhm™ MRSA ACP Lysis Kit package insert and the BD GeneOhm™ MRSA ACP package insert. The same experiment was performed by 4 operators with each of the two types of lysis tube cap (septum and screw caps). The experiment was performed a second time 72 hours later using the original run controls from the first experiment to assess for possible contamination during storage.

No false positive result due to carry-over contamination was observed over a total of 16 runs (8 with septum caps tubes and 8 with screw caps tubes by 4 operators). Storage of the PC and NC tubes at 4°C for 72 hours and the subsequent use in retesting did not lead to invalid runs or carry-over contamination.

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Index of Symbols

SYMBOL	MEANING
	**Manufacturer**
REF	**Catalog number**
	**In Vitro Diagnostic Use**
	**Use by**
	**Contains sufficient for "n" tests**
	**Batch code**
	**Temperature limitation**
	**Protect from light and moisture**
	**Reseal pouches after use**
	**Consult instructions for use**

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