BD MAX™ MRSA

BD MAX™ MRSA Assay REF 442554

For *In Vitro Diagnostic Use*
For use with the BD MAX™ System


INTENDED USE

The BD MAX™ MRSA Assay performed on the BD MAX™ System is an automated 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 real-time 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 MAX™ MRSA 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 OF THE PROCEDURE

MRSA is a major cause of healthcare acquired infections. Most transmissions occur through the contaminated hands of a person carrying MRSA. While MRSA causes infections with clinical manifestations ranging from pustules to sepsis and death[1], it is commonly found in the nose or on the skin of healthy individuals (asymptomatic carriers). Treatment of MRSA infections has become a real challenge with the emergence of strains resistant to a broad range of antimicrobial agents. Methicillin-resistant strains of *S. aureus* are frequently encountered in healthcare settings, and represent over 50% of hospital-acquired *S. aureus* isolates in some North American hospitals[2]. MRSA prevalence continues to increase within many U.S. hospitals and in the community[2,3]. Risk factors for infection with MRSA in healthcare settings include prolonged hospital stay, proximity to patients infected or colonized with MRSA, colonization with other resistant organisms such as Vancomycin-resistant enterococci (VRE) and *Clostridium difficile*, exposure to multiple and/or prolonged broad-spectrum antibiotic treatments, exposure to high MRSA prevalence areas within the healthcare facility, and prior MRSA nasal infection or carriage. Early identification of patients with MRSA nasal carriage can be 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 providing results of MRSA nasal carriage on the day of admission represents a definite advantage for infection prevention programs.

A nasal specimen is collected and transported to the laboratory using the recommended swab (refer to "Equipment and Materials Required But Not Provided" section). The swab is placed in a BD MAX™ MRSA Sample Buffer Tube. The Sample Buffer Tube is vortexed to release cells from the swab into the buffer. The Sample Buffer Tube is placed onto the BD MAX™ System and the following automated procedures occur: the bacterial cells are lysed, DNA is extracted on magnetic beads and concentrated, then an aliquot of the eluted DNA is added to PCR reagents which contain the MRSA-specific primers used to amplify the genetic target, if present. The assay also includes a Sample Processing Control (SPC). The Sample Processing Control is present in the Extraction Tube and undergoes the extraction, concentration and amplification steps to monitor for inhibitory substances as well as process inefficiency due to instrument or reagent failure. No operator intervention is necessary once the clinical sample and reagent strip are loaded into the BD MAX™ System. The BD MAX™ System automates sample lysis, DNA extraction and concentration, reagent rehydration, nucleic acid amplification and detection of the target nucleic acid

sequence using real-time polymerase chain reaction (PCR). Amplified targets are detected with hydrolysis probes labeled with quenched fluorophores. The amplification, detection and interpretation of the signals are done automatically by the BD MAX™ System.

PRINCIPLES OF THE PROCEDURE

The BD MAX™ System uses a combination of lytic and extraction reagents to perform cell lysis and DNA extraction. Following enzymatic cell lysis at elevated temperature, the released nucleic acids are captured by magnetic affinity beads. The beads, with the bound nucleic acids, are washed and the nucleic acids are eluted by heat in Elution Buffer. Eluted DNA is neutralized with Neutralization Buffer and transferred to the Master Mix Tube to rehydrate PCR reagents. The reconstituted amplification reagent is dispensed into the BD MAX™ PCR Cartridge. Microvalves in the BD MAX™ PCR Cartridge are sealed by the system prior to initiating PCR to prevent evaporation and amplicon contamination.

The amplified DNA targets are detected using hydrolysis (TaqMan®) probes labeled at one end with a fluorescent reporter dye (fluorophore) and at the other with a quencher moiety. Probes labeled with different fluorophores are used to detect MRSA and SPC amplicons in two different optical channels of the BD MAX™ System. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of target DNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'-3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the two optical channels used for the BD MAX™ MRSA Assay is directly proportional to the quantity of the corresponding probe that is hydrolyzed. The BD MAX™ System measures these signals at the end of each amplification cycle, and calculation algorithms interpolate automatically the results.

REAGENTS

REF	Contents	Quantity
442554	**BD MAX™ MRSA Master Mix** *Dried PCR Master Mix containing* polymerase, nucleotides and *MRSA specific molecular probe and primers along with Sample Processing Control-specific molecular probe.*	24 tests
	BD MAX™ MRSA Reagent Strips *Reagent strips containing all liquid reagents and disposable pipette tips necessary for specimen processing and DNA extraction.*	24 tests
	BD MAX™ MRSA Extraction Tube *Freeze-dried DNA magnetic affinity beads* *Freeze-dried Achromopeptidase* *Freeze-dried Sample Processing Control*	24 tests
	BD MAX™ MRSA Sample Buffer Tube	24 tests

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BBL™ CultureSwab™ Liquid Stuart single or double swab (Becton Dickinson catalog no. 220099 or 220109), Copan (Venturi) Transystem™ Liquid Stuart single or double swab (Copan, catalog no. 141C or 139C), or
- BBL™ CultureSwab™ Liquid Amies single or double swab (Becton Dickinson catalog no. 220093 or 220105), Copan (Venturi) Transystem™ Liquid Amies single or double swab (Copan, catalog no. 140C or 138C)
- BBL™ CHROMagar™ Staph aureus (BD Diagnostic Systems catalog no. 214982), BBL™ CHROMagar™ MRSA (BD Diagnostic Systems catalog no. 215084), Mannitol Salt Agar (MSA) (BD Diagnostic Systems catalog no. 221773 or 221271) or equivalent media (optional)
- VWR Multi-Tube Vortexer (VWR catalog no. 58816-115)

- NALGENE® Cryogenic Vial holder (VWR, catalog no. 66008-783)
- Gram staining reagent (optional)
- BBL™ Trypticase™ Soy Broth (5 mL) with 6.5% NaCl (BD catalog no. 221351) (optional)
- 5% sheep blood agar plate (e.g. BBL™ Trypticase Soy Agar (TSA II) with 5% Sheep Blood, BD Diagnostic Systems catalog no. 221239 or 221261) (optional)
- Disposable gloves, powderless
- Scissors (optional)
- Gauze
- Stopwatch or timer
- BD MAX™ PCR Cartridges (BD Diagnostic Systems catalog no. 437519)

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective pouches are open or torn upon arrival.
- Close reagent protective pouches promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not remove desiccant from reagent pouches.
- Check reagent strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes) (see Figure 1).
- Check reagent strips to ensure that all pipette tips are present (see Figure 1).
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not use reagents if the foil has been opened or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Do not mix caps between tubes or re-use caps as contamination may occur and compromise test results.
- Do not expose Master Mix and Extraction tubes to alcohol as this may alter bar code readability.
- To avoid contamination of the environment with MRSA amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals in the BD MAX™ PCR Cartridges prevent contamination.
- 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.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the BD MAX™MRSA Assay, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Gloves must be changed before manipulating reagents and cartridges.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in CLSI Document M29[4] and in Biosafety in Microbiological and Biomedical Laboratories[5].
- Wear protective clothing and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.
- Do not pipette 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.

STORAGE AND STABILITY

Collected specimens should be kept between 2 °C and 25 °C during transport. Protect against freezing or exposure to excessive heat.

Specimens can be stored for up to 48 hours at 2-25 °C or up to 5 days at 2-8 °C before testing.

BD MAX™ MRSA Assay reagents and components are stable at 2-25 °C through the stated expiration date. Do not use expired components.

BD MAX™ MRSA Master Mix and Extraction Tubes are provided in sealed pouches. To protect product from humidity, immediately re-seal after opening. Reagent tubes are stable for up to 7 days at 2-25 °C after initial opening and re-sealing.

INSTRUCTIONS FOR USE

Specimen Collection/Transport

Using a recommended swab transport device (refer to "Equipment and Material Required But Not Provided" section), nasal specimens should be collected according to hospital standard operating procedures and/or the following:

1. Moisten the swab(s) with two drops (approximately 50 µL) of sterile physiological saline or use dry.
2. Carefully insert the swab(s) into the patient's nostril (a swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nares).
3. Roll the swab(s) along the mucosa inside the nostril 5 times.
4. Insert the same swab(s) into the second nostril and repeat steps 2 and 3.
5. Replace the swab(s) in its transport tube.
6. Label the transport tube.
7. Transport the swab(s) to the laboratory according to hospital standard operating procedures (Refer to "Storage and Stability" section).

Specimen Preparation

Note: One (1) Sample Buffer Tube, **one** (1) Septum Cap, **one** (1) Master Mix, **one** (1) Extraction Tube and **one** (1) Reagent Strip are required **for each specimen** and **each External Control** to be tested.
Note: For culturing clinical specimens prior to performing the BD MAX™ MRSA Assay, refer to "Culturing of Clinical Specimens" section.

1. Obtain the number of Sample Buffer Tubes corresponding to the number of specimens and external controls to be run.
2. Label each Sample Buffer Tube (clear cap) with the appropriate patient identification making sure not to obscure, write, or label over the barcodes.
3. Remove the cap from the Sample Buffer Tube.
4. Remove the swab from the sample transport tube and place the swab in the corresponding Sample Buffer Tube.
5. Hold the swab by the stem near the rim of the tube (use gauze to minimize risk of contamination). Lift the swab near the liquid level and bend the stem against the edge of the tube to break the swab stem approximately 2-10mm from tube top. Alternative method: use clean scissors to cut the stem.
6. Close the Sample Buffer Tube with a septum cap.
7. Place Sample Buffer Tube in a NALGENE® Cryogenic Vial holder and vortex at maximum speed for one (1) minute with the Multi-Tube Vortexer. Up to 24 samples can be processed simultaneously with the Multi-Tube Vortexer.

BD MAX™ System Operation

Note: Refer to the BD MAX™ System User's Manual for detailed instructions (Operation section).
Note: The BD MAX™ MRSA Assay must be performed immediately after the vortexing step above ("Specimen Preparation", Step 7). If retesting is necessary, revortex sample.
Note: Unreconstituted reagent tubes should be used within 3 hours after being removed from their protective pouch.

1. Turn on the BD MAX™ System and log in by entering **<user name>** and **<password>**.
2. Remove the required number of BD MAX™ MRSA Reagent Strips from the BD MAX™ MRSA Kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes.
3. Remove the required number of MRSA Extraction Tube(s) and MRSA Master Mix Tube(s) from their protective pouches. Remove excess air, and close pouches quickly with the zip seal.

4. For each specimen to be tested, place one (1) BD MAX™ MRSA Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially. Do not skip spaces.
5. Snap one (1) BD MAX™ MRSA Extraction Tube (white foil) into Position 1 of each BD MAX™ MRSA Reagent Strip (see Figure 1).
6. Snap one (1) BD MAX™ MRSA Master Mix tube (green foil) into Position 2 of each BD MAX™ MRSA Reagent Strip (see Figure 1).

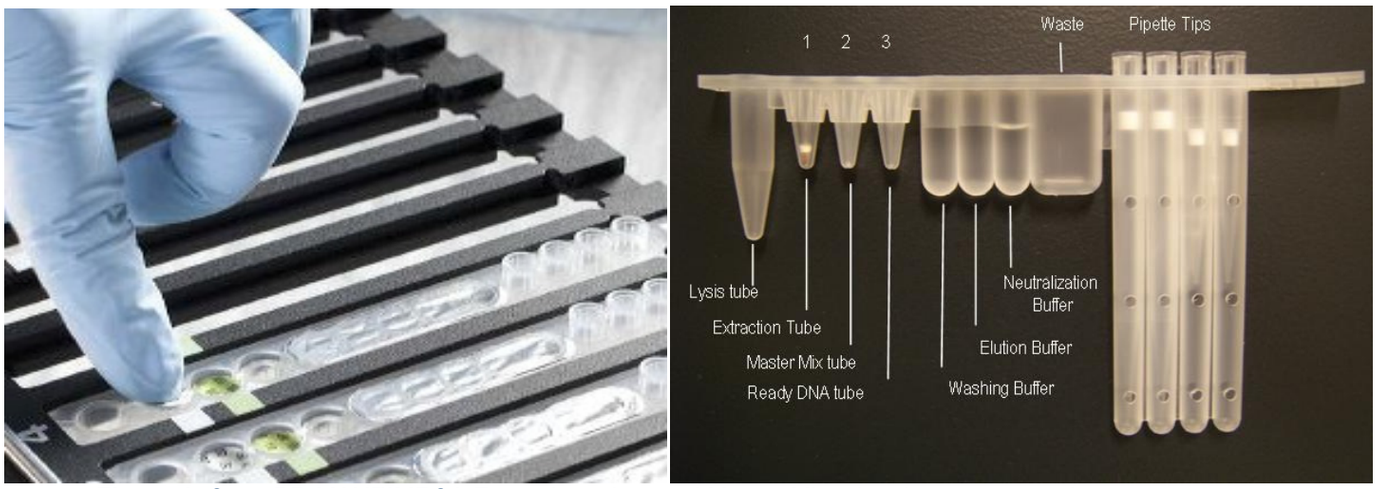

Figure 1: Snap BD MAX™MRSA Extraction tubes and Master Mix tubes into reagent strips

7. To create worklist, select the **<Consumable info>** tab under the *Run* screen on the BD MAX™ software.
8. Enter the kit lot number for BD MAX™ MRSA (for lot traceability) using either the barcode scanner or manual entry.
   Note: Repeat steps 7 and 8 for each new kit lot number.
9. Select the **<Work List>** tab, click on the **<Assay>** field and using the pull down menu, select **<BD MAX MRSA>**.This will automatically fill the remaining position of Rack A with BD MAX MRSA Assay.
10. Enter the BD MAX™ MRSA Sample Buffer Tube ID, and Patient ID or Accession information for Position 1 of Rack A using either the barcode scanner or manual entry.
11. Click on the **<Lot Number>** field and using the pull down menu, select the appropriate box lot number. This will automatically fill the remaining position of Rack A with the same lot number.
12. Enter the information for position 2 in rack A and continue for all remaining Sample Buffer tubes in the rack.
    Note: Steps 11 and 12 must be repeated for each new lot number.
13. Repeat steps 9 to 12 for Rack B.
14. Place the BD MAX™ MRSA Sample Buffer Tube(s) in the BD MAX™ Rack(s) following the same order as entered in the worklist. Do not skip or leave empty positions in between tubes.
15. Place the number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 2). One cartridge is required per rack.
    - Each cartridge is sufficient for up to 2 runs for a total of 24 specimens.
    - The BD MAX™ System will automatically select the position and row on the PCR cartridge for each run.

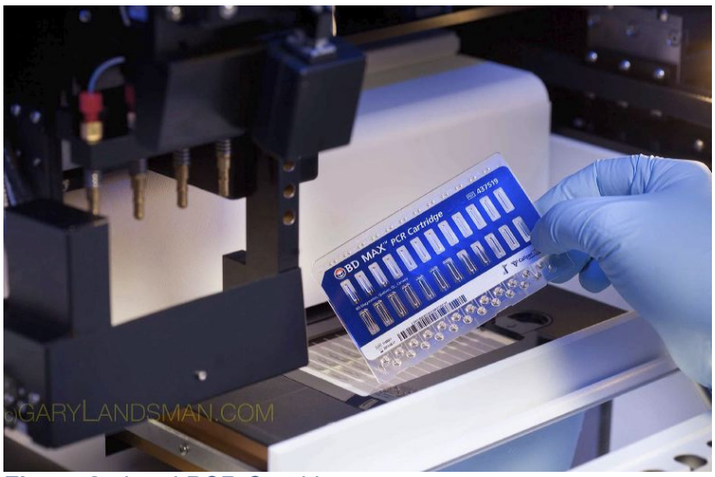

Figure 2: Load PCR Cartridges

16. Load Rack(s) into the BD MAX™ System (Figure 3). Ensure that the placement of Rack(s) (left to right) corresponds to the Work List created (top to bottom).

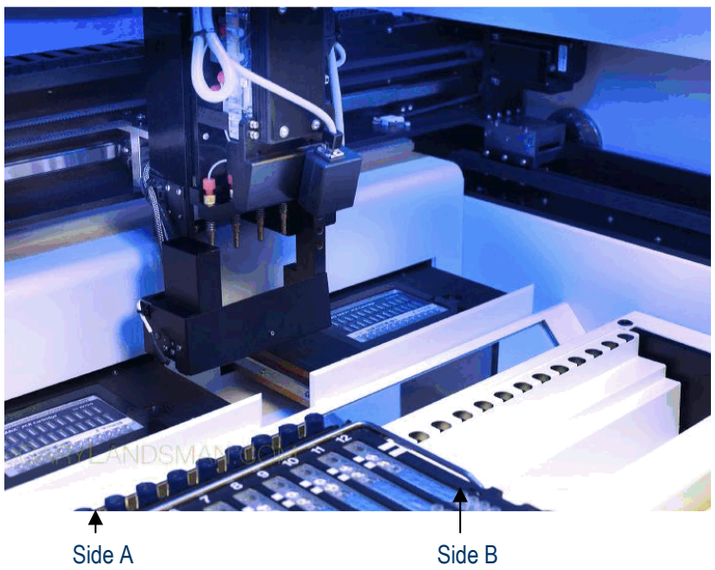

Figure 3: Load Rack(s) into the BD MAX™ System.

17. Close the BD MAX™ System lid and click the <Start Run> button to begin processing.
18. At the end of the run, check results immediately or store Sample Buffer Tubes at 2-8 °C until the results are checked.

Note: If a septum was damaged during the run, replace it with a new one before storing the specimen.

Note: Sample Buffer Tubes can be stored at 2-25 °C for a maximum of 36 hours or at 2-8 °C for a maximum of 120 hours (5 days) after the run has been started. When an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained, or when an External Control failure occurs, a repeat test from the Sample Buffer Tube must be performed within this timeframe (see “Repeat Test Procedure” section).

QUALITY CONTROL

Quality control procedures monitor the performance of the assay. Laboratories must establish the number, type and frequency of testing control materials according to guidelines or requirements of local, provincial, state and/country regulations or accreditation organizations. For general QC guidance, the user may wish to refer to CLSI MM3[5] and C24[6].

1. The External Positive Control is intended to monitor for substantial reagent failure while the External Negative Control is used to detect reagent or environmental contamination (or carry-over) by MRSA amplicons. External Control materials are not provided by BD. Various types of External

Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program:

– Commercially available control materials (e.g. a reference MRSA strain (ATCC 43300) and Methicillin-sensitive *Staphylococcus aureus* (e.g. ATCC 25923) can be used as positive and negative controls, respectively).

– Previously characterized specimens known to be positive or negative for MRSA.

Note: It is recommended that bacterial strains be freshly prepared in saline to a turbidity of 0.5 McFarland (~1.0 X 10^8 CFU/mL) from isolated colonies and subsequently diluted with saline to obtain a final concentration of ~8.0 X 10^3 CFU/mL.

2. One (1) External Positive Control and one (1) External Negative Control should be run daily until adequate process validation is achieved on the BD MAX™ System. Reduced frequency of control testing should be based on adequate data and determined by the individual laboratory.
3. An External Negative Control that yields a positive test result is indicative of a specimen handling and/or contamination problem. Review the specimen handling technique to avoid mix-up and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.
4. An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the "System Error Summary" section of the BD MAX™ System User's Manual[7] for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new BD MAX™MRSA Assay kit.

   Note: External Positive and Negative Controls are not used by the BD MAX™ System software for the purpose of sample test result interpretation. External Controls are treated as if they were patient samples.
5. Each BD MAX™ MRSA Assay Extraction Tube contains a Sample Processing Control (SPC) which is a plasmid containing a synthetic target DNA sequence. The SPC will be extracted, eluted and amplified along with any DNA present in the processed specimen, ensuring the predictivity of the assay. The SPC monitors the efficiency of DNA capture, washing and elution during the sample processing steps, as well as the efficiency of DNA amplification and detection during PCR analysis. If the SPC result fails to meet the acceptance criteria, the result of the specimen will be reported as Unresolved. An Unresolved result is indicative of a high inhibitory specimen or a processing or a reagent failure. Repeat any specimen reported as Unresolved according to the "Repeat Test Procedure" section below.

RESULTS INTERPRETATION

Results are available on the 'Results' tab in the 'Results' window on the BD MAX™ System monitor. The BD MAX™ System software automatically interprets test results. A test result may be called as NEG (negative), POS (positive) or UNR (unresolved) based on the amplification status of the target and of the Sample Processing Control. IND (indeterminate) or INC (incomplete) results are due to BD MAX™ System failure. Results are based on the following decision algorithm.

ASSAY RESULT REPORTED	INTERPRETATION OF RESULT*
POS	MRSA DNA detected
NEG	No MRSA DNA detected
UNR	Unresolved Inhibitory specimen or reagent failure
IND	Indeterminate BD MAX™ System failure (with Warning or Error Codes **)
INC	Incomplete Run (with Warning or Error Codes **)

* BD MAX™ MRSA Assay results may be used to guide isolation and level of precautions in accordance with institutional programs and practices.

** Refer to the "Troubleshooting" section of the BD MAX™ System User's Manual for interpretation of warning and error codes.

REPEAT TEST PROCEDURE

Note 1: Only one repeat is allowed on the BD MAX™ System from the Sample Buffer Tube due to the sample volume available. For Sample Buffer Tubes stored at 2-25 ˚C, retesting must be performed within 36 hours of the steps covered in the "Specimen Preparation" section above. Alternatively, for Sample Buffer Tubes stored at 2-8 ˚C, retesting must be performed within 120 hours (5 days) of the steps covered in the "Specimen Preparation" section above.

Note 2: New samples may be tested in the same run with repeat samples.

UNRESOLVED RESULT

Unresolved results may be obtained in the event that an inhibitory substance prevents proper target, or SPC amplification. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for one (1) minute and restart from the "BD MAX™ Operation" section.

INDETERMINATE RESULT

Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for one (1) minute and restart from the "BD MAX™ Operation" section. For the interpretation of warning or error code messages, refer to the BD MAX™ Software User's Manual[7] ("Troubleshooting" section).

INCOMPLETE RESULT

Incomplete results may be obtained in the event that the Sample Preparation or the PCR did not reach its expected time points. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for one (1) minute and restart from "BD MAX™ Operation" section. For the interpretation of warning or error code messages, refer to the BD MAX™ System User's Manual[7] ("Troubleshooting" section).

EXTERNAL CONTROL FAILURE

External Controls should yield expected results when tested. If specimens have to be repeated due to an incorrect External Control result, they should be repeated from their Sample Buffer Tube along with freshly prepared External Controls within the timeframe defined above. Vortex the samples for one (1) minute and restart from the "BD MAX™ Operation" section".

CULTURING OF CLINICAL SPECIMENS

In order to perform antimicrobial susceptibility testing or epidemiological typing, clinical specimens may be cultured from the collection device (swab) prior to performing the specimen preparation procedure (using the Streak-Plate method) or after the specimen preparation procedure (using the Enrichment Broth method). Swabs may be stored at 2-8°C for up to 36 hours in Sample Buffer Tubes before culturing, following hospital procedures.

LIMITATIONS OF THE PROCEDURE

- This product is intended for use with nasal swab specimens collected using specimen collection and transport devices listed in the "Equipment and Materials Required But Not Provided" section.
- This product should only be used with the BD MAX™ System.
- Negative test results may 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 the BD MAX™ System User's Manual[7] 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 materials and reagents.
- Screening determines the colonization status at a given time. Colonization may vary depending upon patient treatment (e.g. decolonization regime), patient status (e.g. transient MRSA

colonization) or exposure to high-risk environments (e.g. contact with MRSA carrier, prolonged hospitalization). Colonization status should be monitored according to institutional policies.

- A BD MAX™ MRSA positive result does not necessarily indicate eradication treatment failure since DNA presence may persist. A negative result following a previously positive test result may indicate eradication treatment success or may occur due to intermittent colonization.
- A positive test result does not necessarily indicate the presence of viable organisms. A positive result is indicative of the presence of MRSA DNA. The BD MAX™ MRSA Assay simultaneously detects the SCCmec 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 SCCmec/orfX junction of different clinical isolates of MRSA. The MREJ genotype does not correlate with the SCCmec type, i.e., different MREJ genotypes can be associated with each of the known SCCmec types. The BD MAX™ MRSA 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 MAX™ MRSA Assay may not detect other MREJ genotypes, resulting in false negative results.
- The BD MAX™ MRSA 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.
- 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.
- Tobramycin at high concentration may cause slight inhibition in the BD MAX™ MRSA Assay (refer to "Interfering Substances" 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 Sample Processing Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Sample Processing 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 MAX™ MRSA Assay results may sometimes be unresolved due to an invalid Sample Processing Control, or be Indeterminate or Incomplete due to instrument failure, 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 MAX™ MRSA Assay.
- As with all *in vitro* diagnostic tests, positive and negative predictive values are highly dependent on prevalence. BD MAX™ MRSA Assay performance may vary depending on the prevalence and population tested.

EXPECTED VALUES

In the BD MAX™ MRSA Assay clinical study, a total of 913 specimens were tested from 4 geographically diverse U.S. clinical sites by the combined Direct/Enriched culture. The study population was grouped into subjects by the following categories; in-patient or out-patient. The number and percentage of positive cases as determined by this reference method are presented in the table below:

		MRSA By Direct/Enriched Culture		
Group	**Total N**	**Number Positive**	**Number Negative**	**Observed Prevalence**
In-Patient	750	67	683	8.9% (67/750)
Out-Patient	163	9	154	5.5% (9/163)
Total	913	76	837	8.3% (76/913)

PERFORMANCE CHARACTERISTICS

Clinical Performance

Clinical performance characteristics of the BD MAX™ MRSA Assay were determined in a multi-site prospective investigational study. Four (4) 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: 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 excluded.

The Comparative Reference Method consisted of direct culture complemented by enriched culture. Enriched culture analysis was completed for all specimens that were negative for MRSA by direct culture. An initial analysis on a selective chromogenic media was 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 with 5% NaCl (TSB 5%NaCl) was completed in the event that Methicillin-resistant *S. aureus* was not confirmed by the initial method. A turbid TSB 5% NaCl was used to inoculate additional chromogenic media and BA plates, and MRSA confirmation was performed as described above.

A total of 969 nasal swab specimens were tested with both direct culture and the BD MAX™ MRSA Assay. There were 892 reportable results (Tables 1 and 3); 77 nasal swab specimens were excluded from performance analysis due to non-compliance with the clinical study protocol control strategy.

In comparison to the direct culture, the BD MAX™ MRSA Assay identified 97.0% of the MRSA positive specimens and 95.6% of the MRSA negative specimens (Table 2).

Table 1: Results Obtained with the BD MAX™ MRSA Assay in Comparison to Direct Culture

All Sites		Direct culture		
		+	-	Total
BD MAX™ MRSA Assay	+	65	36	101
	-	2	789	791
	Total	67	825	892

Table 2: Performance Obtained using the BD MAX™ MRSA Assay in Comparison to Direct Culture

Clinical Sites	Prevalence	Positive Agreement with 95% CI*	Negative Agreement with 95% CI*
Site 1	2.5% (6/240)	100.0% (6/6) (61.0%, 100.0%)	95.9% (211/220) (92.4%, 97.8%)
Site 2	4.1% (6/147)	100.0% (6/6) (61.0%, 100.0%)	97.2% (137/141) (92.9%, 98.9%)
Site 3	13.1% (29/222)	93.1% (27/29) (78.0%, 98.1%)	95.8% (184/192) (92.0%, 97.9%)
Site 4	8.9% (27/304)	100.0% (26/26) (87.1%, 100.0%)	94.5% (257/272) (91.1%, 96.6%)
Overall	7.4% (68/913)	97.0% (65/67) (89.8%, 99.2%)	95.6% (789/825) (94.0%, 96.8%)

* CI: Confidence Intervals

In comparison to direct/enriched culture, the BD MAX™ MRSA Assay identified 96.0% of the MRSA positive specimens and 96.5% of the MRSA negative specimens (Table 4). For the population tested, this resulted in a Negative Predictive Value (NPV) of 99.6% and a Positive Predictive Value (PPV) of 71.1%.

Table 3: Results Obtained with the BD MAX™ MRSA Assay in Comparison to Direct/Enriched Culture

All Sites		Direct/Enriched Culture		
		+	-	Total
BD MAX™ MRSA Assay	+	72	29	101
	-	3	788	791
	Total	75	817	892

Table 4: Performance Obtained using the BD MAX™ MRSA Assay in Comparison to Direct/Enriched Culture

Clinical Sites	Prevalence	Sensitivity with 95% CI*	Specificity with 95% CI*
Site 1	3.8% (9/240)	100.0% (9/9) (70.1%, 100.0%)	97.2% (211/217) (94.1%, 98.7%)
Site 2	4.1% (6/147)	100.0% (6/6) (61.0%, 100.0%)	97.2% (137/141) (92.9%, 98.9%)
Site 3	13.5% (30/222)	90.0% (27/30) (74.4%, 96.5%)	95.8% (183/191) (92.0%, 97.9%)
Site 4	10.2% (31/304)	100.0% (30/30) (88.6%, 100.0%)	95.9% (257/268) (92.8%, 97.7%)
Overall	8.3% (76/913)	96.0% (72/75) (88.9%, 98.6%)	96.5% (788/817) (94.9%, 97.5%)

* CI: Confidence Intervals

Out of 902 nasal swab specimens tested with the BD MAX™ MRSA Assay, 5 (0.6%) were initially reported as unresolved (Table 5). Upon repeat testing, all had reportable results.

Table 5: Unresolved Rates

Clinical Sites	Initial unresolved rate with 95% CI*		Unresolved rate after repeat with 95% CI*	
Site 1	0.4% (1/228)	(0.1%, 2.4%)	0.0% (0/226)	(0.0%, 1.7%)
Site 2	0.0% (0/148)	(0.0%, 2.5%)	0.0% (0/148)	(0.0%, 2.5%)
Site 3	0.5% (1/222)	(0.1%, 2.5%)	0.0% (0/221)	(0.0%, 1.7%)
Site 4	1.0% (3/304)	(0.3%, 2.9%)	0.0% (0/298)	(0.0%, 1.3%)
Overall	0.6% (5/902)	(0.2%, 1.3%)	0.0% (0/893)	(0.0%, 0.4%)

*CI: Confidence Intervals

Analytical Sensitivity

The analytical sensitivity (Limits of Detection or LoDs) for the BD MAX™ MRSA Assay were determined as follows: Simulated positive specimens were prepared by soaking swabs in a wide range of MRSA bacterial suspensions prepared and quantified from cultures of 6 MRSA strains representing 6 MREJ genotypes (i, ii, iii, iv, v, and vii) and 4 SCC*mec* types (I, II, III, IV). The swabs were then 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 production lots of the BD MAX™ MRSA Assay. Analytical sensitivity (LoD), defined as the lowest concentration at which 95% of all replicates tested positive, ranged from 273 to 645 CFU/swab.

Table 6: Limit of Detection of the BD MAX™ MRSA Assay

MRSA Strain	MREJ Genotype	SCC*mec* Type	LoD Concentration (CFU/swab [95% CI*])
1	type i	I	645 [314,1326]
2	type ii	II	400 [237,678]
3	type iii	III	346 [197,608]
4	type iv	III	490 [264,908]
5	type v	IV	273 [148,504]
6	type vii	II	357 [215,594]

* CI: Confidence Interval

Analytical Inclusivity

A variety of Methicillin resistant *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. Seventy-five (75) strains from 30 countries were tested in this study, including strains from public collections and from well-characterized clinical isolates, including Vancomycin-resistant *Staphylococcus aureus* (VRSA) and Vancomycin-intermediate *Staphylococcus aureus* (VISA) strains.

The BD MAX™MRSA Assay detected all of the MREJ types i, ii, iii, iv, v and vii (wild and mutant) when tested at low bacterial load (2-3 x LoD). The BD MAX™ MRSA 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 Methicillin resistant *Staphylococcus aureus* strains displaying additional resistance to vancomycin (VRSA and VISA) were also detected.

Evaluation of a Well Characterized Challenge Strain Panel

An additional analytical study was carried out to evaluate the analytical performance of the BD MAX™ MRSA Assay using a well characterized challenge strain panel containing the following:

- MRSA strains with high and low Oxacillin minimum inhibitory concentrations (MICs), including PFGE types USA 100, 300, and 400
- BORSA strains (borderline Oxacillin-resistant *S. aureus* strains)
- Methiciilin-sensitive *S. aureus* (MSSA) strains
- Methicillin-resistant *Staphylococcus epidermidis* (MRSE)

The challenge strain panel used in this study was composed of 17 MRSA, 4 BORSA, 1 MRSE and 5 MSSA strains. 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

The BD MAX™ MRSA Assay was performed on samples containing high levels of non-target organisms, using the BD MAX™ System, to demonstrate the specificity of the assay for detection of MRSA.

- Fifty-seven (57) out of 57 strains of various non-staphylococcal species tested at a concentration of 0.5 McFarland produced negative results with the BD MAX™ MRSA Assay.
- Forty-five (45) Coagulase-Negative staphylococci strains (CoNS) and Coagulase-Positive staphylococci strains (CoPS) representing 29 species were tested at a concentration of 0.5 McFarland with the BD MAX™ MRSA Assay. Forty-five (45) of the 45 strains tested exhibited negative results with the BD MAX™ MRSA Assay.
- One hundred-eleven (111) out of 111 MSSA strains tested at extremely high concentrations (> 10^6 CFU/swab), produced negative results with the BD MAX™ MRSA Assay.

Interfering Substances

Twenty (20) biological and chemical substances occasionally used in the nares or found in nasal swab specimens were evaluated for potential interference with the BD MAX™ MRSA Assay. MRSA negative specimens and MRSA positive specimens at 2-3 X LoD were tested 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 Tobramycin that showed slight inhibition (delay of Second Derivative Peak Abscissa) in the BD MAX™ MRSA Assay, however, expected assay results were still obtained.

Table 7: Endogenous and Commercial Exogenous Substances Tested with the BD MAX™ MRSA Assay

Substance	Result	Substance	Result
Mucin, from bovine submaxillary glands	NI	Rhinocort aqua™	NI
Dexamethasone Sodium Phosphate Ophtalmic Solution USP, 0.1% Dexamethasone Phosphate Equivalent	NI	Nasonex™	NI
Chloraseptic™	NI	Fluticasone Propionate	NI
Taro-Mupirocin, Mupirocin Ointment USP, 2%	NI	Luffeel™	NI
Long Lasting Dristan™ Nasal Mist	NI	Zicam® No-Drip Liquid™ Nasal Gel™ Extreme Congestion Relief	NI
Neo-Synephrine™	NI	Relenza™	NI
Otrivin™ Complete Nasal Care™	NI	Tobramycin	*
Beconase AQ™	NI	Blood	NI
Flunisolide Nasal Solution USP, 0.025%	NI	MSSA (ATCC 29213)	NI
Nasacort™ AQ	NI	CNS (ATCC 35983)	NI

NI: No reportable interference with the BD MAX™ MRSA Assay.
*Tobramycin showed slight inhibition (delay of Second Derivative Peak Abscissa) in the BD MAX™ MRSA Assay, however, expected assay results were still obtained.

Precision

Within-laboratory precision was evaluated for the BD MAX™ MRSA Assay at one (1) site. The Precision panel consisted of 4 specimen categories near the LoD. Each specimen 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).

Testing was performed in duplicate, over 12 days, with 2 runs per day, by 2 technologists. Precision study results for Neg, LP and MP samples demonstrated 100% agreement. Precision study results for HN1:100 and HN1:10 demonstrated agreement of 80.6% and 37.5%, respectively.

Reproducibility

The reproducibility study was performed using the same specimen categories as above.

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, LP and Neg categories, 82.2% and 31.1% negative agreement for HN1:100 and HN1:10 categories, respectively (Table 8).

For Lot-to-Lot Reproducibility, the overall percent agreement was 100% for MP, LP and Neg categories, 83.3% and 34.4% negative agreement for HN1:100 and HN1:10 categories, respectively (Table 9).

Second Derivative Peak Abscissa (SDPA), an internal criteria used to determine a final assay result, was selected as an additional means of assessing assay reproducibility. Overall mean SDPA values with variance components (SD and %CV) are shown in Tables 8 and 9.

Table 8: Site-To-Site Reproducibility Study Results using One Lot

Category	SITE						Overall Percent Agreement		SDPA Values[1]		
	Site 1		Site 2		Site 3						
	Percent Agreement		Percent Agreement		Percent Agreement				Overall Mean	SD	%CV
Neg	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95.9%, 100.0%)	31.8	0.47	1.5
HN1:100[2]	22/30	73.3%	27/30	90.0%	25/30	83.3%	82.2%	(73.1%, 88.8%)	32.1	0.85	2.6
HN1:10[2]	12/30	40.0%	3/30	10.0%	13/30	43.3%	31.1%	(22.5%, 41.3%)	31.8	0.48	1.5
LP	60/60	100.0%	60/60	100.0%	60/60	100.0%	100.0%	(97.9%, 100.0%)	31.7	0.66	2.1
MP	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95.9%, 100.0%)	30.4	0.73	2.4

[1] For the Neg category, SDPA values reported are for the SPC. For other categories, SDPA 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.

Table 9: Lot-To-Lot Reproducibility Study Results using Three Lots

Category	LOT						Overall Percent Agreement		SDPA Values[1]		
	Lot 1		Lot 2		Lot 3						
	Percent Agreement		Percent Agreement		Percent Agreement				Overall Mean	SD	%CV
Neg	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95.9%, 100.0%)	31.2	0.76	2.4
HN1:100[2]	26/30	86.7%	24/30	80.0%	25/30	83.3%	83.3%	(74.3%, 89.6%)	31.4	0.79	2.5
HN1:10[2]	6/30	20.0%	12/30	40.0%	13/30	43.3%	34.4%	(25.4%, 44.7%)	31.6	0.71	2.2
LP	60/60	100.0%	60/60	100.0%	60/60	100.0%	100.0%	(97.9%, 100.0%)	31.6	0.77	2.4
MP	30/30	100.0%	30/30	100.0%	30/30	100.0%	100.0%	(95.9%, 100.0%)	30.5	0.66	2.2

[1] For the Neg category, SDPA values reported are for the SPC. For other categories, SDPA 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.

Carryover / Cross-Contamination

A study was conducted to investigate within-run carryover and between-run carryover while processing specimens with high MRSA bacterial load in the BD MAX™ MRSA assay. A panel made of one high positive member and one negative member was used to prepare numerous samples. An MREJ type v MRSA strain was used for the high positive MRSA panel member (8 X 10^7 CFU/swab). The negative member did not contain any target analyte. Twelve (12) replicates of the high positive panel member and 12 replicates of the negative panel member were tested by alternating negative and positive samples. Three (3) operators performed 3 consecutive runs for a total of 9 runs of 24 samples. There were no false positive results due to carry-over contamination.

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BD

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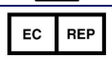
Symbol	Definition
	Temperature limitations
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Authorized Representative
REF	Catalog number
	Protect from light
	Keep dry
	Use by
	Contains sufficient for "n" tests
	Consult instructions for use
	Batch code
	Reseal pouch after use