PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

BD MAX™ MRSA Assay Procedure

Department of Microbiology

Number:

Effective date: **Last Revision:**

Last reviewed:

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1.0 Principle

The BD MAX™ MRSA Assay is an automated qualitative *in vitro* diagnostic test for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs. 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.

A nasal specimen is collected and transported to the laboratory using a swab and transport medium. 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 and placed onto 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. The assay also includes a Sample Processing Control. 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.

2.0 Clinical Significance

MRSA is a major cause of healthcare acquired infections. While MRSA causes infections with clinical manifestations ranging from pustules to sepsis and death, it is commonly found in the nose or on the skin of healthy, asymptomatic individuals. Most transmissions in the healthcare setting occur through the contaminated hands of a person carrying MRSA. Risk factors for infection with MRSA in healthcare settings include prolonged hospital stay, proximity to patients infected or colonized with MRSA, exposure to multiple and/or prolonged broad-spectrum antibiotic treatments, and prior MRSA infection or nasal carriage. Early identification of patients with MRSA nasal carriage can be part of an effective infection prevention program for MRSA. 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.

3.0 Scope

This procedure is classified under CLIA as Moderately Complex. It should be carried out by technical personnel familiarized and trained on all levels of the operation of the BD MAX™ testing platform. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, performance checks, basic troubleshooting, QC checks, administrative tasks and record keeping of information vital to verification of instrument and technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in

the department of continued competence and proficiency on the equipment. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling, storage, and disposal of specimens and items that come into contact with specimens. Place contaminated materials in a biohazardous waste container.

The reagent(s) and/or chemical(s) that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens
- Slightly hazardous reagents

To perform this procedure, you must use:

- Gloves
- Laboratory Coat
- Biological safety cabinet (for specimen processing)

Disinfectant following procedure:

- Bleach dilution sprayers or wipes can be used for on demand disinfectant.
- Ethyl Alcohol (70%)

Reference for spill/decontamination

- MSDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

A nasal swab specimen should be collected and transported to the laboratory using one of the recommended swab transport devices. Specimens may be submitted in the following devices (single or double swab):

- BBL™ CultureSwab™ Liquid Stuart's
- Copan (Venturi) Transystem™ Liquid Stuart's
- BBL™ CultureSwab™ Liquid Amies
- Copan (Venturi) Transystem™ Liquid Amies
- BBL™ CultureSwab™ Plus Amies Gel without Charcoal

Swabs submitted in other transport devices have not been evaluated and should not be used for this assay. Swabs with wire shafts have also not been evaluated for use and should not be used.

5.1 Collection

1. Carefully insert the swab into the patient's nostril. The swab tip must be inserted up to 2.5 cm (1 inch) from the edge of the nares.
2. Roll the swab 5 times.
3. Insert the swab into the second nostril, and repeat sampling.

4. Place the swab in its container, and label the container.

5.2 Transport and Storage

1. Collected specimens should be kept between 2 and 25 °C during transport. Protect against freezing or exposure to excessive heat.
2. Specimens can be stored up to 48 h at 15-25 °C or 5 d at 2-8 °C before testing.

6.0 Materials

6.1 Equipment and/or Testing System

- BD MAX™ System
- Multi-vial vortex

6.2 Consumables

- BD MAX™ PCR Cartridges REF 441770. Store at 2-25 °C
- 4x4s (gauze or alternative)

6.3 Reagents and Media

- BD MAX™ MRSA Assay Kit (BD catalog no. 442953), 24 tests. Store at 2-25 °C.
    - 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 d at 2-25 °C after initial opening and re-sealing.
    - BD MAX™ MRSA Sample Buffer Tubes
    - Septum caps
    - BD MAX™ MRSA Reagent Strips containing the Elution Buffer, Neutralization Buffer and Wash Buffer
- BBL™ Brain Heart Infusion (5 mL) with 6.5% NaCl (BD catalog no. 221785). Store at 2-25 °C.
- BBL™ CHROMagar® MRSA II (BD catalog no. 215229). Store at 2-8 °C.
- BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II™) (BD catalog no. 221261). Store at 2-8 °C.

6.4 Control and Standard Curve Materials and Usage

Suspensions of the control strains should be 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 ~1.0 X 10^4 CFU/mL. Suspensions may be frozen in aliquots at -70 °C and thawed prior to use.

- **Positive External Control:** *Staphylococcus aureus* ATCC 43300
- **Negative External Control:** *Staphylococcus aureus* ATCC 25923

7.0 Interfering Substances

The manufacturer performed studies with the BD MAX™ MRSA Assay in the presence of potential biological and chemical interfering substances in order to characterize the ability of the assay to detect MRSA DNA under these conditions. A complete description of the studies can be found in the manufacture's package insert. Results demonstrated no reportable interference with any substance except for tobramycin that showed slight inhibition in the BD MAX™ MRSA Assay; however, expected assay results were still obtained.

8.0 Warnings and Precautions

- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective foil 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) and that all pipette tips are present (see Figure 1).
- Do not use reagents if desiccant is not present or is broken inside reagent pouches. Do not remove desiccant from reagent pouches.
- 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.
- The seals in the BD MAX™ PCR Cartridges prevent environmental contamination with MRSA amplicons. Do not break apart the BD MAX™ PCR Cartridge after use.
- Performing the assay outside of the recommended time ranges may produce invalid results.
- Gloves must be changed before manipulating reagents and cartridges.
- Wear protective clothing and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.

9.0 Software Instructions

Refer to BD MAX™ System IVD Operation Manual for programming instructions.

10.0 Procedure

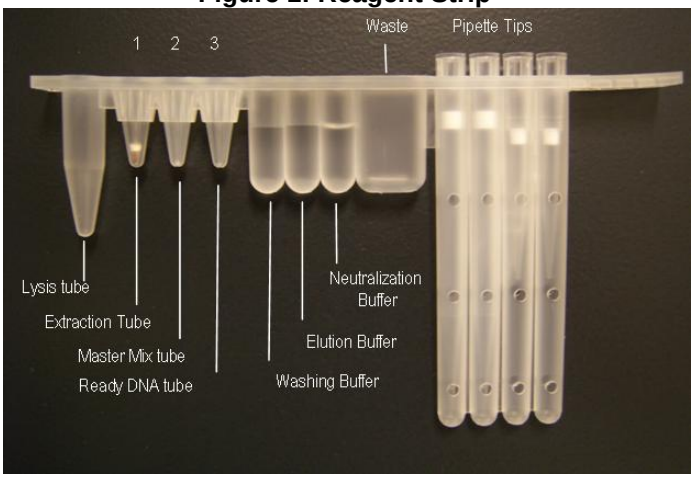
10.1 Specimen Preparation

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 from the batch log, 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, using a 4x4 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 2-10 mm from tube top.
6. Close the Sample Buffer Tube with a septum cap.
7. Repeat process for each specimen, and place Sample Buffer Tubes in a rack. Vortex the rack at maximum speed for 1 min with the Multi-Tube Vortexer.

10.2 BD MAX™ Operation

1. 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.

Figure 1: Reagent Strip

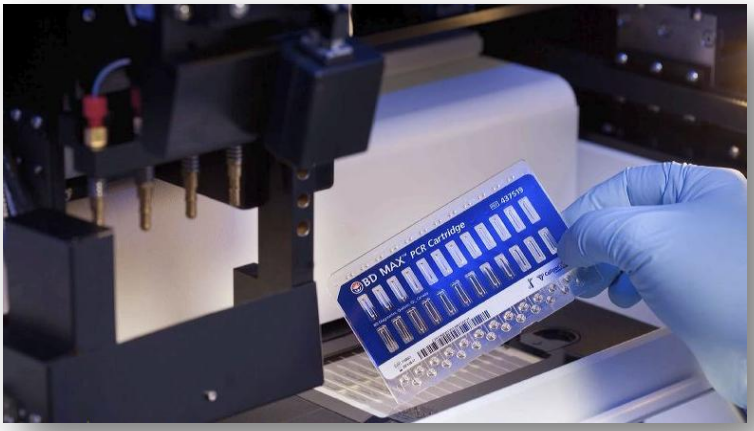


2. For each specimen to be tested, place one BD MAX™ MRSA Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially. Do not skip spaces.
3. Remove the required number of MRSA Extraction Tubes and MRSA Master Mix Tubes from their protective pouches. Remove excess air, and close pouches quickly with the zip seal.
4. Snap one BD MAX™ MRSA Extraction Tube (white foil) into Position 1 of each BD MAX™ MRSA Reagent Strip (see Figure 2).
5. Snap one BD MAX™ MRSA Master Mix tube (green foil) into Position 2 of each BD MAX™ MRSA Reagent Strip (see Figure 2).

Figure 2: Reagent Placement

6. Place the Sample Buffer Tubes into the BD MAX™ System rack so that the number on the tube corresponds to the position on the rack.
7. Select the <Work List> tab, click on the <Assay> field and using the pull down menu, select <MAX MRSA>.
8. 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.
9. Click on the <Lot Number> field and using the pull down menu, select the appropriate box lot number.
10. Enter information for Position 2 of Rack A, and continue until all Sample Buffer Tubes information is entered.
11. Place the number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System (see Figure 3). One cartridge is required per rack per work list. Each cartridge is sufficient for up to 24 specimens and up to 2 work lists. The BD MAX™ System will automatically select the position and row on the PCR cartridge for each run.

Figure 3: PCR Cartridge Placement



12. Load Rack(s) into the BD MAX™ System. Ensure that the placement of Rack(s) (left to right) corresponds to the Work List created (top to bottom).
13. Close the BD MAX™ System lid, and click the <Start Run> button to begin processing.
14. At the end of the run, check results immediately.

11.0 Interpretation and Reporting of Results

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.

Note: 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 h 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 5 d of the steps covered in the "Specimen Preparation" section above.

11.1 Positive Result

A positive (POS) result indicates that MRSA DNA was detected. However, culture should be performed to differentiate between true MRSA and empty cassette strains. Refer to the flow chart on the following page for reporting and follow up testing instructions.

11.2 Negative Result

A negative (NEG) result indicates that no MRSA DNA was detected. A successful negative result is only reported when the Sample Processing Control was amplified and detected.

Report: **Negative for MRSA by PCR**.

11.3 Unresolved Result

Unresolved results may be obtained in the event that an inhibitory substance prevents proper target, or Sample Processing Control amplification. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for 1 min and restart from the BD MAX™ Operation section. If the result is unresolved a second time, report: **Uninterpretable MRSA PCR result. Culture in progress.**

11.4 Indeterminate Result

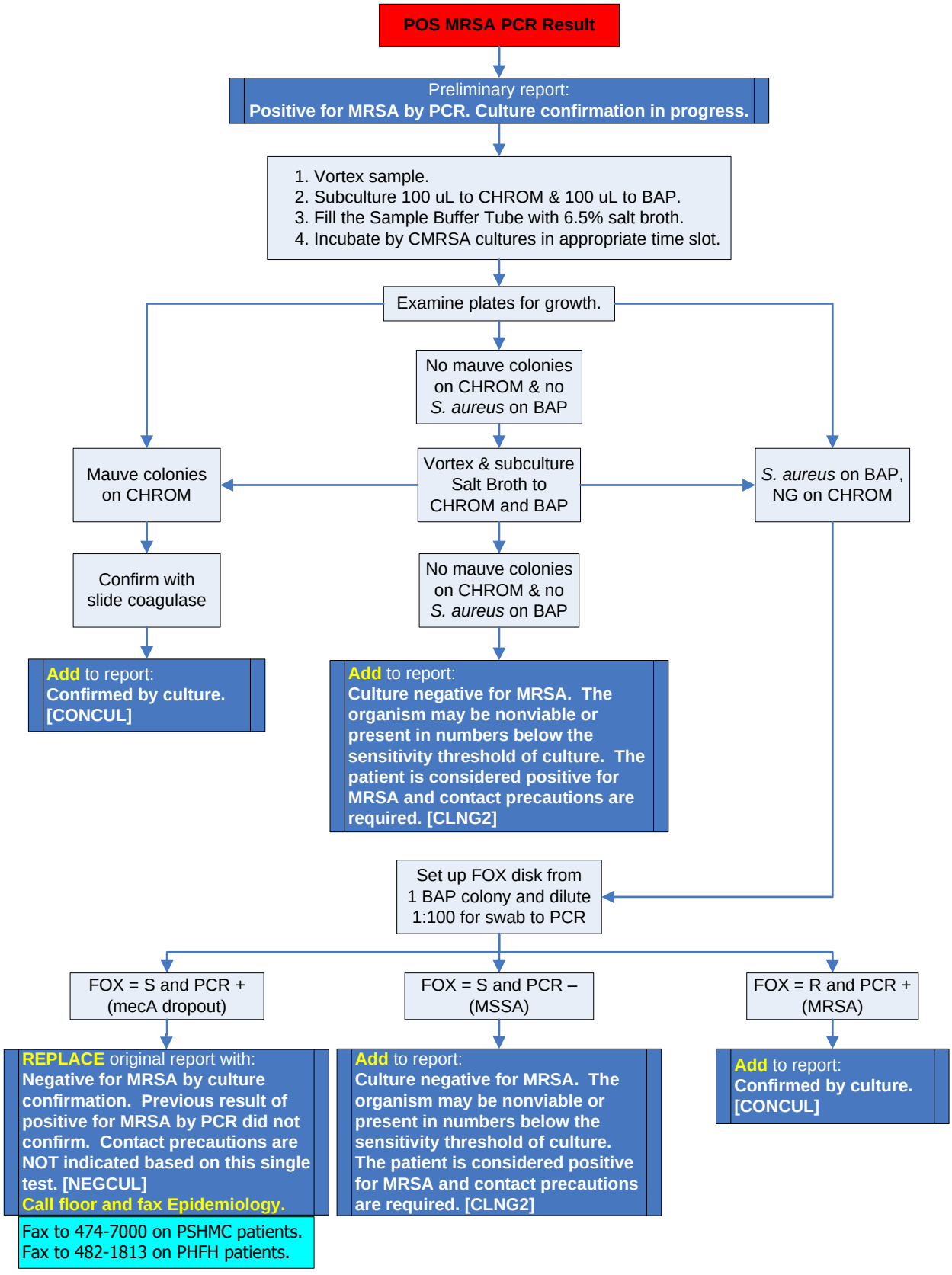
Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from their corresponding Sample Buffer Tube(s) within the timeframe defined above. Vortex the sample(s) for 1 min, and restart from the BD MAX™ Operation section. For the interpretation of warning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

11.5 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 1 min, and restart from BD MAX™ Operation section. For the interpretation of warning or error code messages, refer to the Troubleshooting section of the BD MAX™ System User's Manual.

11.6 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 1 min, and restart from the BD MAX™ Operation section.



12.0 Quality Control & Quality Assurance

12.1 External Controls

External control materials must be used to evaluate each new lot or shipment of BD MAX™ MRSA Assay kits. External controls must be tested every 30 d while a lot is in use. Quality control results should be entered into the LIS. Notify technical specialist or supervisor if results are not as expected. Do not report any patient results obtained from the failed run. Repeat testing using new external controls. Suspensions of the control strains should be 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 (~1.0 X 10^4 CFU/mL). Suspensions may be frozen in aliquots at -70 °C and thawed prior to use.

- **Positive External Control:** *Staphylococcus aureus* ATCC 43300. An external positive control that yields a negative test result is indicative of a reagent or BD MAX™ System error. Repeat Quality Control testing with new controls. Check the BD MAX™ System monitor for any error messages. If the problem persists, use unopened reagents or a new BD MAX™ MRSA Assay Kit.
- **Negative External Control:** *Staphylococcus aureus* ATCC 25923. An external negative control that yields a positive test result is indicative of a specimen handling and/or a contamination problem.

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 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.

12.2 Internal Control

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. 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 specimen-associated inhibition or reagent failure. Repeat any specimen reported as Unresolved.

13.0 Maintenance

13.1 Daily Cleanup

Caution: Do not use any decontamination or cleaning agents that could cause a hazard as a result of a reaction with parts of the equipment. Do not use abrasive or corrosive cleaners on heater boards. Do not spray or pour liquid directly on surfaces.

At the end of each day, perform the following cleaning procedure:

1. Wipe down the following items and areas with disinfecting wipes containing 1% sodium hypochlorite.
   - sample racks (should be cleaned between each run)
   - work surfaces
   - ancillary items such as pipettes, tube racks, etc.
   - all external and internal work surfaces of the BD MAX™ instrument, EXCEPT the monitor screen, the clear part of the instrument door, and the glass surface of the cartridge drawer. External instrument surfaces should be cleaned before internal surfaces.

2. Using a unidirectional motion, thoroughly wipe off all system parts that came into contact with sodium hypochlorite (a known PCR inhibitor) with a lint-free cloth dampened with deionized (DI) water, then with 70% alcohol.
3. Use a new, dampened lint-free cloth for each solution.
4. Dry the system with a lint-free cloth.

13.2 Weekly Cleaning

1. Turn off the BD MAX™ instrument using the On/Off switch.
2. Unplug the BD MAX™ instrument from the Uninterruptible Power Supply (UPS) when performing cleaning and maintenance.
3. Use proper personal protective equipment and follow safety guidelines.
4. Perform routine Daily Cleanup as described above.
5. Inspect the cartridge drawer for foreign objects, dirt, or dust. If any are discovered in the tray, remove and clean the surface with a 70% alcohol solution on a lint-free cloth.
6. Wipe the monitor screen with an alcohol wipe, and then dry the screen with a soft cloth.
7. Use either an alcohol wipe or glass cleaner to clean both the transparent cover of the system and the mirror inside the instrument, using a lint-free cloth to dry.
8. Plug the system back into the UPS and turn on.
9. Put on a clean pair of disposable gloves before beginning instrument operation.

14.0 Instrument Maintenance and Service

14.1 Preventative Maintenance

Preventative Maintenance is performed by a BD field service engineer every 6 months. The engineer checks all of the instrument calibrations and the thermocycler functionality. After the PM is complete, previously tested patient samples should be run to verify the instrument's performance. This should include 5 positive and 5 negative samples for each analyte.

14.2 Service Repairs

If the BD MAX™ instrument malfunctions or operates unusually in any way, initial attempts should be made to solve the problem by following the recommendations in the Troubleshooting section of the System User's Manual. All other servicing attempts will terminate the responsibility of the manufacturer under the terms of the warranty.

If instrument malfunction cannot be corrected, contact BD Technical Services. Technical Services is available Monday through Friday from 5:30 a.m. to 5:00 p.m. Pacific Time. Locate the instrument serial number located on the front of the instrument before placing the call.

Technical Service Information

Telephone Number: 800-638-8663

Email Address: technical_services@bd.com

After major repairs have been made to the instrument, previously tested patient samples should be retested to verify that the instrument is performing as expected.

15.0 Limitations

1. This product is intended for use with nasal swab specimens collected using specimen collection and transport devices listed.
2. 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 are necessary to avoid erroneous results.
3. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.

4. 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).
5. 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.
6. 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.
7. Twenty 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 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.
8. 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.
9. 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.
10. Tobramycin at high concentration may cause slight inhibition in the BD MAX™ MRSA Assay (refer to "Interfering Substances" section for further details).
11. 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.
12. 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.
13. 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.

16.0 Validation Information

The BD MAX™ MRSA Assay has been cleared by the FDA for clinical diagnostic testing. No modifications have been made to the FDA-cleared assay. However, a different collection device with Amies gel instead of liquid Stuart's or liquid Amies transport medium was validated for use. In this evaluation, nasal swab specimens that were tested with the BD GeneOhm™ MRSA Assay were subsequently tested using the BD MAX™ MRSA Assay. The swabs were first eluted into 600 µL of BD GeneOhm™ Sample Buffer, and 90 µL of this cell suspension was used for the BD GeneOhm™ assay. The remaining suspension, approximately 425 µL, was transferred to the BD MAX™ MRSA Sample Buffer tube for analysis. To offset any dilutional effect, 425 µL of the BD MAX Sample Buffer was removed prior to adding the 425 µL cell suspension. The Sample Buffer tubes were vortexed for 1 min, just as they would if they were inoculated with a swab specimen.

Analytical Sensitivity

The Limit of Detection (LoD) of the BD MAX™ MRSA Assay, as determined by the manufacturer, is 273 to 645 CFU per swab. A study was performed to evaluate the LoD using the BBL CultureSwab™ with Amies gel (GEL) in comparison to the recommended BBL CultureSwab™ with Liquid Stuart's transport medium (LIQ). Swabs were seeded with diluted suspensions of *Staphylococcus aureus* ATCC 43300 and then placed into the transport media prior to analysis

with the BD MAX™ MRSA assay. A 0.5 McFarland suspension (approximately 1 x 10^8 CFU/mL) of the MRSA control strain was prepared in saline with the use of a nephelometer. This suspension was serially diluted 1:10 four times to achieve a final suspension with approximately 10^4 CFU/mL. A set of swabs for both transport media types were loaded 100, 50, and 25 µL to produce swabs with approximately 1,000, 500, and 250 CFU. After placing the swabs into their respective transport devices, they were processed and analyzed with the BD MAX™ MRSA assay. All 3 of the LIQ swabs produced positive results while only the 1,000 CFU GEL swab produced a positive result. An additional study was performed to further evaluate the relative performance of the two transport devices. A suspension of the control strain was prepared and diluted to approximately 10^4 CFU/mL as described above. This time, the swabs were seeded with 60 and 30 µL to produce swabs with approximately 600 and 300 CFU. Six replicates of each concentration were prepared for both of the transport devices. The swabs were placed into the transport devices and then processed and analyzed using the BD MAX™ MRSA assay. All 6 of the 600 CFU LIQ swabs produced positive results. Five of the 600 CFU GEL swabs produced positive results with 1 test producing an unresolved result. Five of the 300 CFU LIQ swabs produced positive results, with 1 test unresolved. Four of the 300 CFU GEL swabs produced positive results, with 1 negative and 1 unresolved. These data suggested that the performance of the assay when using the Amies gel is comparable to the performance when using the liquid Stuart's medium recommended by the manufacturer. The data also support the LoD determined by the manufacturer. To see if these results were reproducible, the study was repeated using 6 replicates of 600 CFU and 6 replicates of 300 CFU swabs. This second study produced more variable results. A summary of the results from both runs is illustrated in the table below.

Results from Swabs Seeded with *S. aureus* 43300

	Run 1			Run 2		
	POS	NEG	UNR	POS	NEG	UNR
600 CFU - LIQ	6	0	0	6	0	0
600 CFU - GEL	5	0	1	4	2	0
300 CFU - LIQ	5	0	1	4	1	1
300 CFU – GEL	4	1	1	3	3	0

The variable results that were obtained from the two runs suggested that there might be biological limitations associated with creating and diluting the cell suspensions. To better characterize the comparable performance of the two transport devices, MRSA DNA control material was used to prepare a new set of test swabs. The MRSA DNA was obtained from BD and labeled as a "weak positive." The DNA was reconstituted with 100 µL of diluent per the manufacturer's instructions. The suspension was diluted 1:10 with diluent and used for loading swabs. A set of swabs was prepared for each transport device using 50, 25 and 10 µL. The swabs were inserted into the respective transport device and then processed and analyzed with the BD MAX™ MRSA assay. The 50 and 25 µL LIQ swabs produced positive results, while all 3 of the GEL swabs were positive. The DNA suspension was further diluted 1:10, and another set of swabs was loaded with 50, 25, and 10 µL. In this second run, the 50 µL LIQ swab was positive while the 25 and 10 µL swabs were negative. For the GEL swabs, the 25 µL swab was positive while the 50 and 10 µL swabs were negative. The results from this study indicate that the performance of the assay using the Amies gel is comparable to the assay performance using the liquid Stuart's transport media as recommended by the manufacturer. The table below summarizes the results using the MRSA DNA material.

Results from Swabs Seeded with MRSA DNA

	LIQ	GEL
1:10 Dilution – 50 µL	POS	POS
1:10 Dilution – 25 µL	POS	POS
1:10 Dilution – 10 µL	NEG	POS
1:100 Dilution – 50 µL	POS	NEG
1:100 Dilution – 25 µL	NEG	POS
1:100 Dilution – 10 µL	NEG	NEG

Analytical specificity

The manufacturer of the BD MAX™ MRSA evaluated the assay using samples containing high levels of non-target organisms. Fifty-seven (100%) strains of various non-staphylococcal species tested at a concentration of at least 10^6 CFU/mL produced negative results with the BD MAX™ MRSA Assay. Forty-five Coagulase-Negative staphylococcal strains and Coagulase-Positive staphylococcal strains representing 29 species were tested at a concentration of 0.5 McFarland with the BD MAX™ MRSA Assay. All 45 (100%) of the staph strains tested negative with the BD MAX™ MRSA Assay. One hundred-eleven (100%) MSSA strains tested at extremely high concentrations (> 10^6 CFU/swab) produced negative results with the BD MAX™ MRSA Assay. Seventeen viruses representing 12 different viral species were tested at U 10^5 PFU/mL. All 17 viruses produced negative results with the BD MAX™ MRSA Assay.

Assay Accuracy

A total of 75 nasal specimens were tested in parallel with the BD MAX™ MRSA Assay and the BD GeneOhm™ MRSA Assay. This included 22 samples that tested positive and 53 samples that tested negative on the GeneOhm™ assay. Two (9%) of the positive samples were positive with the GeneOhm™ assay but tested negative with the MAX™ assay. Salt broth enrichment culture was performed on both samples but did not confirm the positive results. However, it was discovered that one of the samples was tested with expired BD MAX™ reagents. The master mix and lysis reagents are only stable for 7 days after opening the seals on the packages. The reagents used for this sample had been open for 11 days. This could account for the discrepant result. The run data for the other sample that produced a discrepant result was submitted to BD for analysis. While no definitive resolution was found, BD did note that the GeneOhm™ amplification curve for the test sample was identical to the curve of the external positive control. A review of the GeneOhm™ test batch showed that the discrepant sample had been placed at the end of the run, following the external controls. It is plausible that the discrepant result was due to cross-contamination or technical error, but this could not be confirmed. Of the 53 samples that tested negative by the GeneOhm™ assay, 1 (2%) of the samples produced a positive result by the MAX™ assay. Salt broth enrichment culture was performed but did not confirm the positive result. A review of the amplification curves revealed very weak responses. The GeneOhm™ curve was suggestive of amplification late in the assay that did not cross the threshold. The MAX™ amplification curve also suggested low level of target. This discrepant result was likely due to a level of target DNA that was close to the LoD for the assays. The table below summarizes the results of the parallel testing for both assays.

	Positive MAX	Negative MAX	Total
Positive GeneOhm	20	2	22
Negative GeneOhm	1	52	53
Total	21	54	75

Overall Agreement = 96%

Positive Agreement = 91%

Negative Agreement = 98%

Precision

A description of the precision studies performed by the manufacturer can be found in the test kit package insert. Our evaluation of the assay's precision consisted of 20 days of Quality Control testing using external control materials. The external positive control was *Staphylococcus aureus* ATCC 43300, and the external negative control was *Staphylococcus aureus* ATCC 25923 grown on BAP. Suspensions of the control strains were 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 (~1.0 X 10^4 CFU/mL). This portion of the verification is currently ongoing.

17.0 References

1. Package insert: BD MAX™ MRSA Assay Kit, 07-2012
2. BD MAX™ System User's Manual BD Diagnostics, Sparks, MD, USA.