PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

BD MAX™ MRSA *XT* Assay Procedure

Department of Microbiology

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1.0 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.

2.0 Principle

The BD MAX™ MRSA *XT* 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. The BD MAX™ MRSA XT assay uses an extended combination of primers and probes to detect new strains of MRSA, including strains with mecA or mecC gene, and decrease false positives due to mecA or mecC dropouts.

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 *XT* 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.

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
- Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System

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 *XT* Assay Kit (BD catalog no. 443460), 24 tests. Store at 2-25 °C.

    - BD MAX™ MRSA *XT* 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 *XT* Sample Buffer Tubes
    - Septum caps
    - BD MAX™ MRSA *XT* 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 Materials

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 *XT* 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 *XT* Assay.

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

10.1.1 ESwab Transport System

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. Briefly vortex the transport devices.
4. Remove the cap from the ESwab transport tube. The swab should be attached to the cap.
5. Remove 250 µL of sample transport tube, and dispense the sample into the corresponding Sample Buffer Tube.
6. Replace the swab and cap on the transport device.
7. Close the Sample Buffer Tube with a septum cap.
8. 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 Vortex.

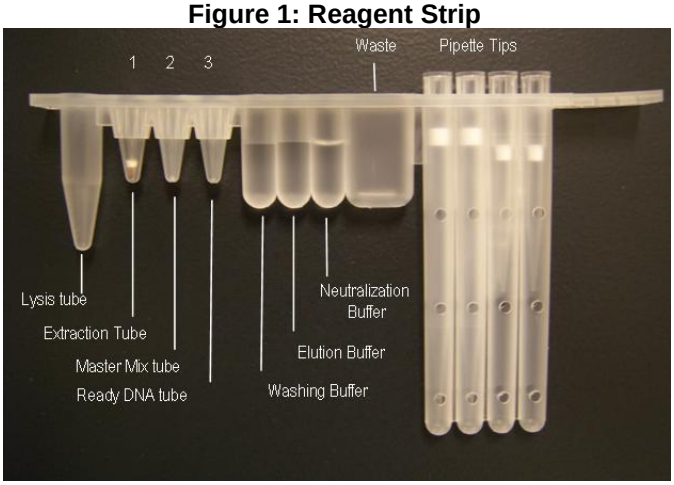
10.1.2 Liquid Stuart's Transport Devices

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 Vortex.

10.2 BD MAX™ Operation

1. Remove the required number of BD MAX™ MRSA *XT* Reagent Strips from the 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

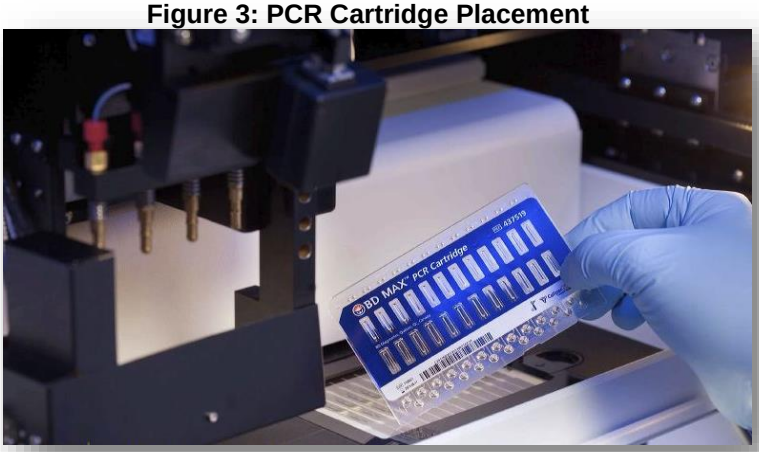


2. For each specimen to be tested, place one MRSA *XT* Reagent Strip on the BD MAX™ System Rack, starting with Position 1 of Rack A and continuing sequentially. Do not skip spaces.
3. Remove the required number of MRSA *XT* Extraction Tubes and MRSA *XT* Master Mix Tubes from their protective pouches.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

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

11.2 Negative Result

A negative (MRSA 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. Replace the previously pierced cap with a new Septum Cap and 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.** Refer to the flowchart below for culture testing.

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. Replace the previously pierced cap with a new Septum Cap and 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. Replace the previously pierced cap with a new Septum Cap and 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.

MRSA UNR PCR Result
Uninterpretable MRSA PCR result.
Culture in progress.

1. Vortex original eSwab sample. For swabs from liquid Stuart's, vortex the sample buffer tube.
2. Subculture 100 uL to CHROM.
3. Incubate by CMRSA cultures in appropriate time slot.

Examine plates for growth

Mauve colonies on CHROM

Confirm with slide coagulase

Culture results:
(MRSA) Staphylococcus aureus Resistant microorganism.
Contact precautions required.

No mauve colonies on CHROM

Culture results:
No MRSA Isolated.

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 *XT* 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. When the control material is used for testing, the final concentration of organisms is approximately 1 X 10^3 CFU/swab. This is close to the manufacturer's published limit of detection of 64 to 343 CFU/swab. The weak positive control material helps to verify the lower limit of detection for each new lot/shipment. 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 *XT* 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 *XT* 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 *XT* 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.

12.3 Report Review

All test results entered into LIS should be reviewed by a second technologist on the same shift or the beginning of the next shift. Results should be compared to the printed results from the BD MAX™ computer. The review should be documented on the MRSA PCR Batch Log.

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. Perform routine Daily Cleanup as described above.
2. 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.
3. If necessary, wipe the monitor screen with an alcohol wipe, and then dry the screen with a soft cloth.
4. 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.
5. 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 *XT* 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 *XT* assay simultaneously detects the *mecA or mecC* gene carried within the SCC*mec* cassette and a *S. aureus* specific sequence located within the junction of the SCC*mec* cassette and the *orfX* gene (MREJ).
7. The BD MAX™ MRSA *XT* assay is designed to detect MREJ genotypes i, ii, iii, iv, v, vi, vii, ix, xiii, xiv and xxi which represents most of *mecA* and *mecC* harboring MRSA strains (belonging to different SCC*mec*/MREJ types) accounting for more than 98% of worldwide strains tested by BD to date. The ability of BD MAX™ MRSA XT assay to detect other MREJ genotypes is unknown.
8. The BD MAX™ MRSA *XT* assay does not report Borderline Oxacillin Resistant *S. aureus* (BORSA) as MRSA (it will report as NEG). The mechanism of oxacillin resistance in BORSA strains is due to an increased production of β-lactamases, not the *mecA* or *mecC* gene. BORSA strains are rare.
9. The BD MAX™ MRSA *XT* assay performance in detecting modified *S. aureus* (MOD-SA) is not known as those strains have not been evaluated. The mechanism of oxacillin resistance in MOD-SA strains is due to changes in affinity of penicillin-binding proteins for oxacillin. MOD-SA strains are rare.
10. The BD MAX™ MRSA *XT* assay will generate a false positive MRSA result when testing a nasal specimen containing both a methicillin-resistant coagulase negative *Staphylococcus* (MRCoNS) and an "empty cassette" methicillin-susceptible SA variant. Co-colonization with MRCoNS and an “empty cassette” methicillin-susceptible SA is rare.
11. 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.
12. Tobramycin may interfere with the BD MAX™ MRSA *XT* assay (refer to “Interfering Substances” section for further details).
13. 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 and as a control for reagent integrity and of the assay system as a whole. 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.
14. In a mixed culture, the detection of MRSA is variable when high concentrations of MRSE are present. Competition from MRSE was observed at an MRSA:MRSE ratio of 1: ≥ $1x10^3$.
15. In a mixed culture, the detection of MRSA is variable when high concentrations of MSSA are present. Competition from MSSA was observed at an MRSA:MSSA ratio of 1: ≥ $1x10^4$.
16. BD MAX™ MRSA *XT* 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 obtaining final results.
17. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown MRSA, resulting in a false negative result with the BD MAX™ MRSA *XT* assay.

16.0 Verification Information

Accuracy

Verification of the BD MAX™ MRSA *XT* assay was initially performed using diluted suspensions of 12 *S. aureus* clinical isolates, 14 previously characterized *Staphylococcus* isolates, and 12 nasal specimens that previously tested positive with the BD MAX™ MRSA assay.

The 12 clinical *S. aureus* isolates included 10 "empty cassette" strains that previously produced false-positive results with the BD MAX™ MRSA assay. The other two isolates were MRSA strains that had previously been missed by other testing methods. One isolate produced a false-negative MRSA result with the BD GeneOhm Staph SR assay, and the other MRSA isolate was previously incorrectly identified as MSSA by the BD Phoenix instrument. Isolate suspensions were prepared by harvesting overnight growth from a BAP and creating a suspension equivalent to a 0.5 McFarland standard using Phoenix ID broth and the Phoenix AP instrument (~1.0×10^8 CFU/mL). These suspensions were serially diluted with saline to a concentration of ~1.0×10^4 CFU/mL. A 100 µL aliquot of each suspension was loaded onto swabs, producing a final concentration of ~1.0×10^3 CFU/swab. The swabs were placed into Amies gel transport medium and processed and tested by following the manufacturer's protocol. All 10 (100%) of the clinical *S. aureus* empty cassette strains were correctly reported as negative by the BD MAX™ MRSA *XT* assay. The two clinical isolates previously misidentified by the BD GeneOhm Staph SR and the BD Phoenix were correctly identified as MRSA by the BD MAX™ MRSA *XT* assay.

The 14 previously characterized *Staphylococcus* isolates were comprised of 1 *S. aureus* mecA dropout (BD 3097), 1 *S. aureus* MSSA (ATCC 29213), 1 *S. epidermidis* (ATCC14990), 1 *S. aureus* mecC (ATCC BAA 2312), and 10 *S. aureus* mecA variants. These isolates were obtained from BD as lyophilized microorganisms on swabs and used directly for testing with the BD MAX™ MRSA *XT* assay. All 14 of the previously characterized *Staphylococcus* isolates were correctly identified. Table 1 summarizes the details and results from the isolate testing.

Table 1: Testing results for previously characterized *Staphylococcus* strains.

Isolate ID	Strain type	mrej type	MRSA XT	Expected MRSA Result
S. aureus BD 3097	MSSA mecA dropout	ii	NEG	NEG
S. aureus BD 1	MRSA	i	POS	POS
S. aureus BD 2800	MRSA	ii	POS	POS
S. aureus BD 9	MRSA	iii	POS	POS
S. aureus BD 11	MRSA	iv	POS	POS
S. aureus BD 16	MRSA	v	POS	POS
S. aureus BD 2937	MRSA	vi	POS	POS
S. aureus BD 19	MRSA	vii	POS	POS
S. aureus BD 131	MRSA	ix	POS	POS
S. aureus BD 2952	MRSA	xiii	POS	POS
S. aureus BD 797	MRSA	xiv	POS	POS
S. aureus ATCC BAA 2312	MRSA (mecC)	xxi	POS	POS
S. aureus ATCC 29213	MSSA	N/A	NEG	NEG
S. epidermidis ATCC 14990	MSSE	N/A	NEG	NEG

The 12 nasal swab specimens that tested positive by the BD MAX™ MRSA assay were submitted in Amies gel transport medium. The swabs were eluted in sample buffer and tested according to the BD MAX™ MRSA protocol. The residual eluted specimens were tested using the BD MAX™ MRSA *XT* assay. The remaining eluted samples were cultured overnight using Brain Heart Infusion with 6.5% NaCl and subcultured to BBL™ CHROMagar® MRSA II and BBL™ Trypticase™ Soy Agar with 5% Sheep Blood. Eight (67%) of the specimens tested positive by both PCR assays and were confirmed by culture. Three (25%) of the specimens were positive by the BD MAX™ MRSA assay only and not confirmed by the BD MAX™ MRSA *XT* assay or by culture. One (8%) sample was positive by both PCR assays but did not grow MRSA in culture. An empty cassette strain was recovered from the culture of this specimen. Testing of this isolate produced a false-positive BD MAX™ MRSA result and a negative BD MAX™ MRSA *XT* result.

While no performance issues were encountered using specimens in Amies gel transport medium during the initial verification studies, subsequent testing using clinical samples produced an unresolved rate of 7%. The positivity rate did not appear to be adversely affected. Additional studies were performed, with the help of a BD molecular application specialist, to determine the cause of the increased unresolved rate. The BD specialist determined that the magnetic beads were not adequately captured during the DNA extraction process. Some of the beads were getting into the microfluidic PCR cards and clogging the channels. BD technical services confirmed that the magnetic beads in the *XT* assay were different than those used in the previous BD MAX MRSA assay. The new beads were adversely affected by the gel transport medium. The only acceptable transport medium listed in the *XT* package insert is liquid Stuart's. However, a collection device with liquid Stuart's was not currently in use within the hospital system. Additional validation studies were performed to confirm that eSwab collection devices containing liquid Amies transport medium could be used with the *XT* assay.

ESwab Validation

A total of 24 paired nasal swabs were self-collected from volunteers in the microbiology lab using eSwab collection devices. The specimens were vortexed, and 200 µL of each eluted sample was transferred to sample buffer tubes and tested with the BD MAX™ MRSA and the BD MAX™ MRSA *XT* assay. One of the specimens tested positive for MRSA by both assays and was confirmed by culture on CHROMagar™ MRSA II. The remaining 23 nasal specimens were seeded with 23 different clinical MRSA isolates. Isolate suspensions were prepared by harvesting overnight growth from a BAP and creating a suspension equivalent to a 0.5 McFarland standard using Phoenix ID broth and the Phoenix AP instrument (~1.0×10^8 CFU/mL). These suspensions were serially diluted with saline to a concentration of ~1.0×10^4 CFU/mL. A 100 µL aliquot of each suspension was added to the eSwab devices, producing a final concentration of ~1.0×10^3 CFU/eSwab. The spiked specimens were vortexed, and 200 µL of each eluted sample was transferred to sample buffer tubes and tested with the BD MAX™ MRSA and the BD MAX™ MRSA *XT* assays. Initially, 22 of the spiked specimens tested positive with the BD MAX™ MRSA assay, and 20 of the spiked specimens tested positive with the BD MAX™ MRSA *XT* assay. Two of the spiked specimens produced unresolved results with the *XT* assay that were positive upon repeat testing. One of the spiked samples produced negative results with both assays. A new sample was prepared using the same isolate with another nasal specimen. The newly prepared spiked sample produced a positive MRSA result.

Table 2: Performance of the BD MAX™ MRSA vs. the BD MAX™ MRSA *XT* assay using eSwab specimens (Note: 2 eSwab specimens initially unresolved).

	MRSA NEG	MRSA POS	Total
MRSA XT NEG	23	0	23
MRSA XT POS	0	23	23
Total	23	23	46

Additional testing was performed to compare the performance of the BD MAX™ MRSA *XT* assay using paired liquid Stuart's and eSwab devices seeded with low concentrations of CFU/swab. *S. aureus* ATCC 43300 was used for the positive test strain. A suspension of the control strain was prepared in saline to a turbidity of 0.5 McFarland (~1.0×10^8 CFU/mL) from isolated colonies growing on BAP. The suspension was serially diluted and used to inoculated paired swabs with ~2.0×10^3, 1.0×10^3, and 5.0×10^2 CFU/swab. The seeded swabs were placed in their respective transport devices and allowed to sit for 15 min prior to testing. The liquid Stuart's swabs were tested as described in the package insert. For the eSwab samples, the transport devices were vortexed, and then 200 µL of the transport medium was transferred to a sample buffer tube for testing. Table 2 summarizes the results.

Table 2: Comparison of eSwab to Liquid Stuart's transport devices (Note: 1 eSwab specimen was unresolved).

CFU/Swab	Liquid Stuart's			eSwab		
	POS n(%)	NEG n(%)	UNR n(%)	POS n(%)	NEG n(%)	UNR n(%)
2,000	4(67)	0	2(33)	6(100)	0	0
1,000	6(100)	0	0	6(100)	0	0
500	12(100)	0	0	8(67)	3(25)	1(8)

These data suggest that there may be a slight decrease in the sensitivity when using the eSwab transport device. A recent study[3] published in the Journal of Clinical Microbiology also found that the use of the eSwab device may diminish the sensitivity of the BD MAX™ MRSA *XT* assay, as compared to using liquid Stuart's transport medium. However, the difference was not statistically significant. Furthermore, the flocked swabs used in the eSwab transport device are known to yield superior samples with more cellular material due to the increased surface area of the swab. The flocked swabs also readily release specimen from the collection device rather than it being trapped in woven fibers of conventional swabs.

Additional studies were performed to determine how much of the liquid Amies could be used from the eSwab device for performing the assay. Testing was performed using *S. aureus* ATCC 43300. A suspension of the control strain was prepared in saline to a turbidity of 0.5 McFarland (~1.0×10^8 CFU/mL) from isolated colonies growing on BAP. The suspension was serially diluted and used to inoculated eSwab devices with ~1.0×10^3 and 1.0×10^2 CFU/swab. The seeded swabs were placed in their respective transport devices and allowed to sit for 15 min prior to testing. The devices were vortexed, and the assay was performed using 250, 500, and 700 µL of the liquid Amies. Table 3 summarizes the results.

Table 3: Impact of sample size of eSwab liquid Amies specimen used for testing

CFU/Swab	250 µL			500 µL			700 µL		
	POS n(%)	NEG n(%)	UNR n(%)	POS n(%)	NEG n(%)	UNR n(%)	POS n(%)	NEG n(%)	UNR n(%)
1,000	25(93)	2(7)	0	5(19)	19(70)	3(11)	0	6(100)	0
100	11(41)	16(59)	0	1(4)	26(96)	0	0	4(67)	2(33)

The optimal volume of liquid Amies sample was 250 µL. At 500 and 700 µL there was a high rate of false negative and unresolved results. No unresolved results were obtained when using 250 µL of sample from the eSwab devices.

Overall, the combined rate of unresolved results with the BD MAX™ MRSA *XT* assay when using 200-250 µL of liquid Amies was 2% (3 out of 124 verification samples).

Precision

Precision studies were performed by testing external control materials for 20 days. 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 (~1.0×10^8 CFU/mL) from isolated colonies and serially diluted with saline to obtain a final concentration of ~1.0×10^4 CFU/mL. When the control material is used for testing, the final concentration of organisms is approximately 1×10^3 CFU/swab. This is close to the manufacturer's published limit of detection of 64 to 343 CFU/swab. Testing was performed by 12 different users. All of the external controls provided the expected results.

17.0 References

1. Package insert: BD MAX™ MRSA *XT* Assay Kit, 01-2014
2. BD MAX™ System User's Manual BD Diagnostics, Sparks, MD, USA.
3. Dalpke AH, Hofko M, Stock C. Evaluation of the BD MAX MRSA *XT* Assay for Use with Different Swab Types. *J. Clin. Microbiol.* 2014;vol. 52 no. 12:4343-4346.

18.0 Document Control History

Reviewed by director (AR) 06/11/2013, 07/01/2014, 12/03/2014

Reviewed by supervisor (JC) 06/11/2013, 07/02/2014, 12/03/2014, Jason Ammons 12/2015

Changes and updates:

05/30/2013 Added Frequency of Testing under Specimen Information.

06/07/2013 Updated validation section for precision after 20 successful days of testing with external control material.

05/13/2014 Updated information regarding positive external control concentration. The control material is prepared to be weak and close to the LOD.

07/01/2014 Updated procedure for the BD MAX™ MRSA *XT* assay.

12/03/2014 Updated for testing using eSwab transport devices.