# BD MAX™ MRSA Assay Procedure

**PURPOSE**

* This procedure provides instructions for preparing samples and testing on the BD MAX™ instrument

#### POLICY STATEMENT

* MRSA PCR testing is performed 1 – 2 times daily, 0700 –1530
* **Alert Value: PMRSA** **(first time positive patients) -** Report results by telephone to the patient’s caregiver; document

**ABBREVIATIONS**

|  |  |
| --- | --- |
| * MRSP: methicillin-resistant *S. aureus* PCR * IC: internal control * INC: incomplete run * IND: indeterminate * LIS: laboratory information system * MM: master mix * MREJ: SCC *mec* right extremity junction * NA: nucleic acid * NEGC: negative control | * PCR: polymerase chain reaction * POSC: positive control * pp: primer – probe * RT – Room temperature * UNR: unresolved   Area/Room 1: Clean room  Area/Room 2: Processing room  Area/Room 3: Amplification room |

## DOCUMENTATION/RECORDS

* BD MAX run-specific Results Report
* LIS Incomplete and Completed worksheets
* Daily Maintenance Log

## SAFETY CONSIDERATIONS

* Standard precautions. Refer to MB 2.02 Biohazard Containment
* Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 2   * Refrigerator 2 – 8° C * -70⁰ C freezer * VWR Multi-tube vortex * BSC BSL-2   Room 3   * + BD MAX instrument | BD MAX MRSA Assay kit: Product No. 443461 | Orange barrier wipes |
| MRSA positive control | Nitrile gloves (powder-free) |
| MSSA negative control | BD MAX PCR cartridges |
| Sani-Cloth Bleach wipes | Nalgene cryogenic vial holder |
| 70% alcohol | BD MAX Sample rack |
| water | Septum Caps |

#### MATERIALS REQUIRED

## QUALITY CONTROL

1. Assay Controls
   1. A POSC and NEGC must be included in each assay run.
   2. An IC is incorporated into each extraction tube

1. QC Monitors:

|  |  |
| --- | --- |
| **Control** | **Control Monitor** |
| MRSA Positive Control (POSC) | Cell lysis, reagent failure and primer-probe integrity |
| MSSA Negative Control (NEGC) | Reagent and/or environmental contamination, cumulative effect |
| Internal Control (IC) | PCR inhibition in specimen, reagent failure or process error |

1. Before reporting patient results, all controls must yield valid results. Refer to MB 10.05, Procedures F and G, Evaluating and Interpreting Results.

**PROCEDURE A:** Follow the steps in the table below to organize and label samples, prepare specimen for testing

Specimen Preparation

| **Activity** | | Step | | **Action** | **Related Doc** |
| --- | --- | --- | --- | --- | --- |
| ***Note:*** *Create worklist and load strips in racks prior to vortexing step #15. Refer to MRSA 005 Procedures B, C* | | | | | |
|  | 1 | | Call worksheet **MRSP**; use this worksheet for sample identification throughout testing. | | MB 1.01  Specimen Management |
| **Sample Organization**  Room 2 | 2 | | Process up to 22 patient samples plus one POSC and NEGC per run. Position samples and controls as follows:   |  |  | | --- | --- | | Sample | Position | | Patient samples | 1 – nn | | POSC ( **+** ) | 2nd to last position | | NEGC ( **-** ) | Last tube | | | MB 3.01 Engineering Controls |
|  | 3 | | Using the **MRSP** worksheet as a layout, organize patient samples and labels   * Color code worksheets and labels per run * Number patients on worksheet in consecutive order * Number patient labels including 1 aliquot label corresponding to worksheet | |  |
| **Numbering**  **BufferTubes and swabs** | 4 | | Number and label one buffer tube per patient sample, POSC and NEGC   * Place Sample buffer tubes in rack * Number each cap and tube consecutively * Place aliquot label on corresponding tube, matching number to the cap * Do not cover barcode | |  |
|  | 5 | | Number each patient swab according to MRSP worksheet | |  |
|  | 6 | | Place swabs in the rack behind the corresponding tube | |  |
| **Processing**  **Room 2** | 7 | | Loosen caps on each sample buffer tube, allowing the caps to sit lightly on tube   * *Only one tube can be open at a time* | |  |
|  | 8 | | Remove test swab from the sample transport tube | |  |
|  | 9 | | Lift cap from corresponding tube and discard | |  |
|  | 10 | | Place swab into the sample buffer tube | |  |
|  | 11 | | Break swab off in sample buffer as follows:   * Using an orange barrier protector, hold the swab near the rim of the tube * Lift the swab up 1 cm from the bottom of the tube * Bend the swab against the edge of the tube to break * Return swab shaft to original transport tube * Discard barrier protector | |  |
|  | 12 | | Place septum cap on tube, touching only the edges to secure | |  |
|  | 13 | | Repeat steps 8 – 12 for all samples to be tested | |  |
|  | 14 | | Change gloves | |  |
| **Vortex**  Room 2 | 15 | | Vortex tubes in rack for 1 minute (Speed 10, Timer 10)   * Place protective barrier on top of septum caps prior to vortexing * *Perform assay immediately after vortexing step, Procedure D* | | MB 10.05  Procedure D: *Running MRSA Assay* |
| Room 3 | 16 | | Remove lab coat; move to room 3 with test samples | |  |

**PROCEDURE B:** Follow the steps in the table below for logging samples into the system

Log in Sample Tubes

| **Activity** | Step | **Action** | | **Related Doc** |
| --- | --- | --- | --- | --- |
| **Log In** | 1 | **Log In** to the system |  | [BD MAX User Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212335.pdf) |
|  | 2 | Enter username and password  \*\**Case sensitive* |  |  |
| **Work List** | 3 | Click **Run** button |  |  |
|  | 4 | Click the **Work List** tab (see Figure 1) | |  |
| **Method of entry** | 5 | Click on position A1; an arrow will appear to the right of the position column (see Fig. 1)   |  |  | | --- | --- | | To set up Work list | Then | | Row by row | Click arrow to point | | Column by column | Click arrow to point | | |  |
| **Select Assay** | 6 | Click the **Assay** field in row A1 | |  |
|  | 7 | Select **BD** **MAX MRSA XT** from drop down box; all Rack A fields will populate | |  |
| Figure 1: Work List Screen | | | | |
| **14**  **5** | | | | |
|  | 8 | **Sample Tube Barcode** field: Scan Sample buffer tube barcodes just prior to starting run, *Procedure D* | |  |
| **Assigning test position** | 9 | Click in the **Patient ID** field and type in the assigned position number 1 – nn corresponding to MRSP worksheet/label/tube | |
|  | 10 | Click in the **Accession** field and scan the corresponding barcode label   * Controls: type in MRSA/MSSA followed by date as a unique identifier, i.e., MRSA 09.13.16 | |  |
|  | 11 | Select positive or negative from the drop down box in the **External Control** field to designate type of control | |  |
| **Lot Number** | 12 | In the **Lot Number** field, select the lot number in use from the drop down box to populate all rows in Rack A  ***Note:*** *If second lot is necessary, note location and scan into the work list* | | MB 10.10  Creating Lot Numbers in BD MAX |
|  | 13 | To continue in **Rack B,** click on position B1 for the remaining samples   * Refer to steps 7 – 12 | |  |
| **Clearing information for (and below) a selected line** | 14 | To clear information, click the desired row once; an “ **X**” appears right of the Position field | |  |
| 15 | Click the “**X**” button twice to clear information in that row and the rows below | |
|  | 16 | If additional samples are added, re-select the assay and lot numbers | |  |

**PROCEDURE C:** Follow the steps in the table below for preparation of reagents and racks

BD MAX™ prepare racks

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Activity** | Step | **Action** | | Related Doc |
|  |  | ***Note:*** *For optimal performance, run samples in multiples of 4 when possible* | |  |
| **Gloves** | 1 | Clean gloves must be used for handling all reagents | |  |
|  | 2 | Remove the required number of reagent strips from the MRSA kit. | |  |
| **Reagent strips**  Room 3 | 3 | Position strips in the sample rack according to work list   * Place the pipette tips toward the front * Seat the back of the strip under the back rail of the sample rack and front edge under the front lip * Pull strip back slightly to make sure it is seated properly | |  |
|  | 4 | Tap rack on a hard surface to ensure all liquids are at the bottom of the tubes | |  |
| **Extraction and MM tubes** | 5 | Snap the MRSA extraction tube into position 1 (white) of each reagent strip | Figure 2. Snap in reagents  **6 - green**  **5 - white** |  |
| 6 | Snap the MRSA MM tube into position 2 (green) of each reagent strip |
| 7 | Reseal securely each reagent pouch containing desiccant and remaining tubes |
|  | 8 | Use MM and extraction reagents within 3 h after removal from pouch | |  |

**PROCEDURE D:** Follow the steps in the table below for running the BD MAX MRSA assay

**Running the BD MAX MRSA assay**

| **Activity** | Step | **Action** | | | | **Related Doc** |
| --- | --- | --- | --- | --- | --- | --- |
| Room 3 | 1 | Place Sample Buffer Tubes into the Sample Rack in the assigned positions  *Note: Face buffer tube barcodes outward for scanning* | | | |  |
|  | 2 | Click in the **Sample Tube Barcode** field on worklist | | | |  |
|  | 3 | Place tubes into Racks A and B following the same sample order as the work list | | | |  |
|  | 4 | Click on **Unlock Door** button, push down on lid gently and lift to open   * The door is unlocked for 15 seconds; after 15 seconds the door relocks | | | |  |
| **Sample Racks** | 5 | Place sample racks into system   * Rack A on left, Rack B on right * The racks have pegs to guide and secure rack in place * Lower handle to the back of the rack once in place | | | |  |
| **Insert Cartridges** | 6 | Place PCR cartridges into the cartridge drawer   * Position the notched edge into the upper left corner * Make sure the cartridge lies snuggly on the reader drawer * Cartridges can be used 2X | | Figure 3. Inserting cartridge | |  |
| **Begin Run** | 7 | Close the door | | | |  |
|  | 8 | Click the **Start Run** button | | | |  |
| **Initialize** | 9 | Instrument will perform a catalog check, reading barcodes on reagent strips and tubes | | | |  |
| **Catalog check errors** | 10 | The **Status** screen automatically appears as the run begins. If there are any errors in the catalog check, the alert icon flashes red and a red alert icon appears for that consumable   * Run will stop * Click on flashing icon to display message and to acknowledge the alert   **Alerts** button information   * Blue: no active alerts * Flashing red: active *unacknowledged* alerts * Red: active *acknowledged* alert | **Figure 4. Catalog errors** | | |  |
| Run  Status | 11 | **Alerts Display**   * Expand alert field to get text describing the alert, click thebutton  * Persistent errors must be corrected  * Non-persistent errors can be cleared from the display | | | **Figure 5. Alerts Display** |  |
|  | 12 | Catalog errors must be corrected before the run can continue | | | |  |
|  | 13 | Click the unlock door to correct error | | | |  |
| **Correct Catalog Errors** | 14 | Correct source of error (examples)   * Replace cartridge * Missing reagent tube * Swapped extraction and PCR reagents * Sample buffer tubes in wrong location | | | |  |
|  | 15 | Once corrected, close door and click **Start Run** to resume testing   * *Warning: never try to open door when the “Unlock Door” is grayed out* | | | |  |
|  | 16 | Monitor Status display for successful catalog check   * All reagents and strips are green | | | |  |
| **Run icons**   * **Rack** * **Robotic pipettor** * **PCR** | 17 | **Figure 6:** Monitoring Run Status while in progress  **Reader A | Robot | Reader B**    **Rack PCR/Reader**   * Rack icon: rack icon may be level, tilted to the left or right, or grayed out if rack not present. If tilted, an alert icon will appear over the rack * Robotic pipettor & PCR icons: when active, moving circles appear; alerts display above the icon | | | |  |
| **Run Completed** | 18 | Run time approx. 2 h | | | | MB 10.11 Interleaved Runs |
| 19 | Log off when testing is completed | | | |  |

**PROCEDURE E:** Follow the steps in the table below for rack removal and sample storage

Rack removal and sample storage

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| **Status** | 1 | Racks can be remove when:   * The run is completed ***or*** * The robot status is idle and the PCR/reader status displays a moving circle   **Figure 7. Status Messages**  Status messages in light blue bars |  |
| **Completed** |
|  | 2 | Click on **Unlock Door** button, open door |  |
|  | 3 | Remove racks containing reagent strips from the run |  |
| Room 3 | 4 | Remove reagent strips from racks and place in a Ziploc bag; dispose bag in red biohazard container in room 3 |  |
|  | 5 | Positive patient samples: Replace septum cap with screw cap; hold in designated rack in refrigerator for 1 month in room 2 |  |
|  | 6 | Hold negative sample buffer tubes in room 3 organized by Run until end of day; if no problems identified, discard tubes in room 3 |  |
|  | 7 | Remove PCR cartridges if used 2X and place in Ziploc bag for disposal |  |
|  | 8 | If another run can be performed on the cartridge, load the new sample racks on the instrument deck |  |
|  | 9 | If there are no more runs, store cartridges in instrument until next run (good for 7 days) |  |
| **Cleaning** | 10 | Clean room and equipment; refer to Decontamination procedure for schedule | MB 10.08  Equipment and Room Decontam |

**PROCEDURE F:** Follow the activities below for PCR Analysis and Printing Results

PCR Analysis and Printing Results

| **Activity** | **Step** | **Action** | | **Related Doc** |
| --- | --- | --- | --- | --- |
|  | 1 | Click the Results button ; the run list appears, Fig. 8 | |  |
|  | 2 | Any runs in progress will display the run icon | |  |
|  | 3 | Click the desired run from the list | |  |
|  | 4 | Click the Print button; the print preview window appears, Fig. 9 | |  |
|  | 5 | Wait for the *Print Preview* to fully populate | |  |
|  | 6 | Click Print for report and graphs  |  |  | | --- | --- | | Analyte | Instrument Channel | | MREJ (*Staphylococcus aureus* specific) | 475/520 | | Methicillin resistance gene (*mec* A or *mec* C) | 585/630 | | Internal control | 680/715 | | |  |
|  | 7 | Change gloves and lab coat before leaving room 3 | |  |
| **Figure 8: Results List** | | | **Figure 9: Print Preview** | |

**PROCEDURE G:** Follow the activities below for evaluating the acceptability of patient results

Evaluating and Interpreting QC and Patient Results

| **Activity** | **Step** | | **Action** | | **Related doc** |
| --- | --- | --- | --- | --- | --- |
|  | 1 | | Check QC for acceptability before reporting patient results | |  |
| QC and Patient acceptability | 2 | | If | Then | [BD MAX User Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212335.pdf)  7.2 System Problems  7.4 Error list  MB 10.06 Trouble-shooting Guide  Refer to Procedure H: Repeat Testing |
| Valid assay: Controls as expected | * Report patient results |
| Invalid assay:  POSC/ NEGC fail | * Do not report patient results * Review the sample handling/ preparation technique * Repeat testing using failed control and a new control |
| NEGC positive | * Do not report patient results * Review the sample handling/ preparation technique * Review specimen mix-up or contamination * Repeat testing, refer to Procedure H * *Tech spec/director review required – If positive patient in failed run* *and not a previous positive,* *recollect patient sample* |
| External control: Unresolved, Indeterminate or Incomplete result | * Failure caused by reagent or system failure * Check for error messages * Repeat testing * Problem repeats, refer to trouble-shooting guide |
|  |  | | Problem unresolved | * Call BD technical service, **1-800-638-8663**, option #2 * Notify Technical Specialist |  |
| Problem | | 3 | Record problem/action in the QC failure log. | |  |
| Interpretation | | 4 | The system software will automatically interpret the specimen results; Refer to Table 1 | | Refer to Table 1 and  MB 10.07  For Reporting |
| **Results Interpretation and entry** | | 5 | Enter results in Sunquest Microbiology Result Entry using Culture Mode | |
|  | | 6 | Alert Value: PMRSA (first time positive patients) - Report results by telephone to the patient’s caregiver; document | |  |
| **Review** | | 7 | Call completed MRSP worksheet to review reporting. | |  |
| **Archive** | | 8 | Initial LIS result worksheet and SC run specific report. | |  |
|  | | 9 | Place reports in MRSA result log book. | |  |

###### Table 1: Interpretation of results – For additional information, refer to [MB 10.07](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/MRSA/212323.pdf) *Reporting and Archiving Results* and

MB 10.05, Procedure H: *Repeat Testing*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample Type** | **Test Result** | **Sunquest Code** | **Repeat testing** | **Interpretation of result** |
| Patient Samples |  | NMRSA |  | No MRSA DNA detected, MRSA nasal colonization unlikely   |  |  |  |  | | --- | --- | --- | --- | | Target | MREJ | *mec* A/ *mec*C | IC | | NMRSA | - | - | + | | NMRSA (*mec* A / C drop out) | + | - | + | | NMRSA (S. epi) | - | + | + | |
|  | PMRSA |  | MRSA DNA detected, MRSA nasal colonization   |  |  |  |  | | --- | --- | --- | --- | | Target | MREJ | *mec* A/ *mec*C | IC | | NMRSA | + | + | NA | |
| UNR | UNR | √ | Unresolved – inhibitory specimen or reagent failure   * No target or IC amplification * If sample remains UNR, call caregiver for new collection |
| IND | ICLA or EQ | √ | Inconclusive results, repeat testing [free text: to be performed on *date*]   * Sample was indeterminate or had a LLS error; an instrument error occurred for this sample * Refer to [BD MAX User Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212335.pdf) for interpretation of error codes * Repeat testing * If sample remains IND, report EQ (equivocal result) |
| INC | ICLA or EQ | √ | Inconclusive results, repeat testing [free text: to be performed on *date*]   * Sample prep or PCR did not meet expected time limits * Refer to [BD MAX User Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212335.pdf) for interpretation of error codes * Repeat testing * If sample remains INC, report EQ (equivocal result) |
| POSC |  | ------- |  | Valid POSC; valid run when NEGC is also valid. |
|  | ------- | √ | Invalid POSC; invalid run. Patient results cannot be reported. |
| NEGC |  | ------- |  | Valid NEGC; valid run when POSC is also valid |
|  | ------ | √ | Invalid NEGC; invalid run. Patient results cannot be reported. |

INC – Incomplete run; IND – Indeterminate; UNR – unresolved

**PROCEDURE H:** Follow the activities below for repeat testing

**Repeat Testing**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| Type of Failure | 1 | For repeat testing, refer to following table for appropriate sample. Repeat testing can be performed one time from Sample buffer tube.  |  |  |  |  | | --- | --- | --- | --- | | Failure | Sample Buffer Tube | Recollect New sample | New Controls | | Negative POSC | √ and |  | √ | | Positive NEGC | √ and |  | √ | | UNR | √ | √ if 2X |  | | INC | √ | √ if 2X |  | | IND | √ | √ if 2X |  | | POSC is negative: repeat all patient samples | √ |  |  | | NEGC is positive: patient is positive | Previous positive  √ | Newly identified positive  √ |  | | NEGC is positive: patient is negative | √ |  |  | | MB 10.06 Troubleshooting  [BD MAX User Manual](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212335.pdf)  7.2 System Problems  7.4 Error list |
| **Timeframe** | 2 | Retesting from RT sample buffer tube must be performed within 36 hours after the end of the run or within 5 days if stored at 2 – 8⁰ C |  |
|  | 3 | Repeat samples may be retested in the same run as new samples |  |
| **Vortex** | 4 | Vortex the sample buffer tube 1 min prior to retesting |  |
| **Error Codes** | 5 | For interpretation of error codes or warnings, refer to Troubleshooting guide |  |

#### METHOD PERFORMANCE

* Clinical Sensitivity/Specificity: 93% / 95.9%

**PROFICIENCY TESTING**

* WSLH PT – MR: MRSA/VRE screen

#### ALTERNATE METHOD

1. MRSA Culture/Surveillance MRSA Culture
2. Sunquest Order code: MRSS, SMRSS
3. Logistics:
   1. Acceptable specimen: Anterior nares
   2. Transport:

* BD CultureSwab
* RT

## LIMITATIONS

1. A positive test result does not necessarily indicate the presence of viable organisms.
2. This assay does not detect the *mec*A gene directly or 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.
3. This assay may cross-react with some strains of MSSA when they are present in extremely high concentrations.
4. Excess blood in the specimen may inhibit the assay.
5. Low levels of target below the LoD of the assay may be detected, but results may not be reproducible.

**REFERENCES**

1. BD MAX™MRSA Assay, Product Circular Reference 443461, P0167(04), 2016-02, GeneOhm Sciences Canada, Inc, 2555Boul. Du Parc Technologique, Quebec (QC), G1P 4S5, Canada
2. BD MAX™ System User’s Manual (US IVD Version), 2014-06 Document Number: 8089572(01), Becton Dickinson and Company, 7 Loveton Circle, Sparks, Maryland 21152 USA

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| --- | --- | --- | --- | --- |
| **Historical Record** | | |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Pat Ackerman | 6/10/2010 | Initial Version |
| 2 | P. Ackerman | 12.12.13 | Updated with BD MAX assay information |
| 3 | P. Ackerman | 5.08.14 | Added Procedure H: Repeat testing |
|  | 4 | P. Ackerman | 09.14.2016 | Reformatted for CMS upload; updated logo; revised table 1; updated screen shots; renumbered prev MRSA 005 |
|  | 4 | J. Laramie | 09.14.2016 | Biennial review: 04.05.2018, JL |
|  | 5 | J. Laramie | 09.03.2018 | Updated alert value notes to specify first time patients only |