PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

MRSA Screen Culture Procedure

Department of Microbiology

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1.0 Clinical Significance

Methicillin-resistant *Staphylococcus aureus* (MRSA) pose a significant risk for causing nosocomial infections, especially in certain patient populations. Infection with MRSA typically follows colonization. Transmission in the healthcare setting primarily occurs via the hands of healthcare workers that are contaminated from contact with colonized or infected patients. If proper hand hygiene is not performed, the bacteria may spread and colonize subsequent patients. MRSA infection occurs most frequently among patients who undergo invasive medical procedures or who have weakened immune systems. MRSA in healthcare settings commonly causes serious and potentially life-threatening infections, such as bloodstream infections, surgical site infections, or pneumonia. The identification of colonized patients may aid in the prevention of MRSA transmission from person to person. The MRSA Screen is a culture specifically targeted at the isolation and identification of this pathogen so that healthcare workers can initiate contact precautions.

2.0 Principle

BBL™ CHROMagar™ MRSA II is a selective and differential chromogenic medium for the qualitative direct detection of nasal colonization by methicillin-resistant MRSA to aid in the prevention and control of MRSA in healthcare settings. BBL CHROMagar MRSA II medium permits the direct detection and identification of MRSA through the incorporation of specific chromogenic substrates and cefoxitin. MRSA strains will grow in the presence of cefoxitin and produce mauve colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are incorporated for the suppression of gram-negative organisms, yeast and some other gram-positive cocci. Bacteria other than MRSA may utilize other chromogenic substrates in the medium resulting in the growth of colonies that are not mauve.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiar with and trained to perform and interpret clinical cultures. Testing includes but is not limited to: culture interpretation, confirmatory testing, Quality Control testing, and record keeping.

4.0 Safety - Personal Protective Equipment

Performance of this procedure may expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

To perform this procedure, you must use:

- Gloves – must be worn when handling specimens.
- Laboratory Coat – must be worn when handling specimens, cultures, and reagents.
- Biological Safety Cabinet – must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

5.0 Specimen Collection, Handling and Storage

Although a variety of sites can be used to screen for MRSA colonization, nasal specimens are preferred.

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 into bacterial transport medium and label the container.
5. Specimens should be kept at 2 to 30°C during transport. Protect against freezing or exposure to excessive heat

Studies were performed in-house to validate the use of CHROMagar™ MRSA II with wound swabs and positive blood culture bottles that are growing gram-positive cocci resembling staph.

6.0 Materials

6.1 Equipment and/or Testing System

- Aerobic incubator set at 35 ± 2°C

6.2 Consumables

- Sterile inoculating loops
- Sterile swabs

6.3 Media & Reagents

- BBL™ CHROMagar™ MRSA II - On receipt, store plates in their original sleeve wrapping and box at 2 – 8°C until time of inoculation. Prolonged exposure to light (> 4 h) may result in reduced recovery and/or coloration of the QC strains or patient isolates. Plates may be used until the expiration date. Avoid freezing and overheating. Allow the medium to warm to room temperature before inoculation.
- Staph latex reagent or coagulase reagent

7.0 Procedure

7.1 Specimen Processing

1. Inoculate a small area of the agar surface at the edge.
2. Streak the plate for isolation.

7.2 Culture Incubation

Incubate plates aerobically at 35 ± 2°C for 18 to 26 h in an inverted position (agar-side up). Do not incubate in an atmosphere supplemented with carbon dioxide. CHROMagar plates should not be incubated beyond the 26 h time period prior to reading.

7.3 Culture Workup

1. After incubation, examine the culture for growth consistent with MRSA.
2. Confirm isolates growing on CHROMagar MRSA II with a slide coagulase test.

8.0 Interpretation & Reporting of Results

8.1 MRSA Isolated

On CHROMagar MRSA II, MRSA appear as mauve-colored colonies. If the isolate is confirmed by slide coagulase, report:

- **(MRSA) Staphylococcus aureus [MRSA]**
- **Resistant microorganism. Contact precautions required. [RESOR1]**

8.2 No MRSA Isolated

If no *S. aureus* is isolated from the culture, report:

- **No MRSA isolated [NMRSA]**

9.0 Quality Control

Each new lot or shipment of CHROMagar MRSA II should be examined for product deterioration and tested with the following control strains. Prepare a 0.5 McFarland suspension of each test strain and dilute 1:10. Use a 0.01 mL calibrated loop to inoculate the media. Incubate plates at 35 ± 2°C in an aerobic atmosphere.

Control strain	Expected Results
S. aureus ATCC 43300	Mauve colonies
S. aureus ATCC 29213	No growth or non-mauve colonies

10.0 Limitations

1. Minimize exposure (< 4 h) of BBL CHROMagar MRSA II to light both before and during incubation, as prolonged exposure may result in reduced recovery and/or coloration of isolates.
2. Keep plates within the original sleeve wrapping and box for the entire storage period.
3. MRSA concentrations of lower than 10^6 CFU/mL may yield false negative results on BBL CHROMagar MRSA II.
4. At 24 h, some strains of *Chryseobacterium meningosepticum*, *Corynebacterium jeikeium*, *Enterococcus faecalis* (VRE), *Rhodococcus equi*, and *Bacillus cereus* may produce mauve-colored colonies. If colony morphology is atypical, a Gram stain may be performed.
5. Resistance mechanisms other than *mecA* (i.e., borderline oxacillin-resistant *Staphylococcus aureus*-BORSA, and modified *Staphylococcus aureus*-MODSA), have not been extensively evaluated with BBL CHROMagar MRSA II, therefore the performance of BBL CHROMagar MRSA II with such resistance mechanisms is unknown.
6. The growth requirements of certain strains of MRSA can lead to their partial or complete inhibition in culture.
7. Incubation in CO_2 is not recommended and may result in false negative cultures.
8. A heavy bacterial load and/or some specimens may produce nonspecific coloring of the primary quadrant of the medium. This could result in the medium exhibiting mauve, purple, green or blue coloration or a slight haze on top of the medium, but lacking distinct colonies. Non-specific coloring of the medium should not be interpreted as positive

11.0 Verification Information

In this evaluation, BBL™ CHROMagar™ MRSA II (CHROM) was compared to the currently used Spectra™ MRSA agar by Remel (SPECTRA). To evaluate the performance of these media, a total of 69 nasal samples that previously tested PCR-positive for MRSA were used. A 100 µL sample of each sample buffer suspension was plated onto a CHROM, SPECTRA, and a BBL TSA plate with 5% sheep blood (BAP). These plates were incubated aerobically 18 - 26 h. The BAP cultures grew various combinations of *S. aureus*, coagulase-negative staph, diphtheroids, alpha-strep, beta-strep, coliforms, and *Pseudomonas*. SPECTRA plates were examined for blue colonies consistent with MRSA and CHROM plates were examined for mauve colonies. Suspect colonies were confirmed by slide coagulase with Staphaurex (Remel). A total of 45 (65%) of the nasal specimens yielded MRSA on SPECTRA. Forty-nine (71%) of the specimens yielded MRSA on CHROM. The additional specimens that yielded MRSA only on CHROM produced only 1 or 2 colonies and were likely at the sensitivity threshold for culture. Four (6%) of the specimens did not yield MRSA from direct plating but were positive for MRSA after selective enrichment with 6.5% NaCl broth. Seven (10%) other specimens yielded *S. aureus* on BAP only. These isolates were evaluated further with the assistance of BD and were determined to be *mec*A drop-out strains and not true MRSA. The 8 (12%) remaining specimens did not yield *S. aureus* on any media by direct specimen planting or after salt broth enrichment.

The second phase of this evaluation included wound specimens that were submitted for bacterial culture. A total of 10 specimens that yielded MRSA in routine culture were retrieved and used to inoculate CHROM. These plates were incubated aerobically overnight. All 10 (100%) of the specimens yielded mauve colonies consistent with MRSA. Inoculum from the CHROM plates

was used to perform AST on Phoenix PMIC-108 panels. These results were compared to those obtained from the routine cultures. No minor or major discrepancies were encountered on isolates tested from CHROM as compared to routine media.

In February, 2012, a study was completed to evaluate the use of CHROMagar™ MRSA II with instrument positive blood cultures that were smear-positive for gram-positive cocci resembling staph. A total of 78 positive blood cultures, representing 52 different patients, were subcultured to CHROM. This included 25 cultures positive for MRSA, 25 cultures positive for MSSA and 28 cultures positive for coagulase-negative staph. The CHROM plates were inoculated with one drop from each blood culture and streaked for isolation. The plates were incubated aerobically overnight at 35 ± 2°C and examined for growth. The results from the CHROM cultures were compared to the results obtained from conventional testing for each isolate. Isolates were routinely identified by gram stain morphology, colony morphology, and slide coagulase. Antimicrobial susceptibility testing was performed using Phoenix PMIC-108 panels. Cefoxitin disk diffusion was used for coagulase-negative staph isolates.

All 25 (100%) of the cultures with MRSA produced growth with expected colony morphology. None (0%) of the MSSA isolates grew on the CHROM medium. Twelve (43%) of the coagulase-negative staph cultures were cefoxitin resistant. These isolates grew on the CHROM medium but did not produce colonies that would be confused with MRSA. The colony morphology of the methicillin-resistant coagulase-negative staph isolates varied from a clear haze to white colonies. None of them were pink or as large as the MRSA colonies after 24 h of incubation. The other 16 (57%) of the coagulase-negative staph isolates were cefoxitin susceptible and did not grow on the CHROM medium at all.

Based on the results of this study, CHROMagar™ MRSA II medium would serve as a reliable method to reduce the time to identification of staph isolates as MRSA vs. MSSA by at least 24 h. This could be useful in guiding optimal antimicrobial therapy. However, for the shortened identification time to be relevant, results of either MRSA or MSSA should be phoned to the clinician at the time of identification.

12.0 References

1. Package insert: BBL™ CHROMagar™ MRSA II, L010089, Rev. 01, September 2010.
2. Center for Disease Control. Laboratory detection of Oxacillin/Methicillin-resistant *Staphylococcus aureus*. February 2, 2005. Available at: http://www.cdc.gov/ncidod/dhqp/ar_lab_mrsa.html

13.0 Document Control History

Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010.

Reviewed by Microbiology Director, Dr. Ann Robinson: 12/04/2009, 02/28/2011

Reviewed by Microbiology Supervisor, Jerry Claridge: 12/04/2009, 03/2011, 03/2013, Jason Ammons 05/2015

Revisions & Updates: 02/23/2011 Changed medium from Spectra MRSA (Remel) to CHROMagar MRSA II (BD). 04/12/2012 Added verification summary for using medium with blood cultures. 02/05/2015 Deleted: “For sites other than upper respiratory, perform a Phoenix PMIC panel using isolated MRSA colonies.”