PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Staphylococcus Screen (MRSA & MSSA) Culture Procedure

Department of Microbiology

Effective date: 05/12/2014

Last Revision: 05/06/2014

Last reviewed: 05/06/2014

Table of Contents

1.0 Clinical Significance

Staphylococcus aureus is a leading cause of surgical site infections (SSIs). Patients with nasal colonization of *S. aureus* have an increased probability of developing SSIs. Identifying colonized patients permits treatment to eliminate nasal carriage prior to surgery to help prevent SSIs.

2.0 Principle

Culture screening for *S. aureus* includes differentiation between methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) isolates. CHROMagar™ MRSA II is used as a selective medium to isolate MRSA. MRSA isolates are confirmed by slide coagulase. TSA II with 5% Sheep Blood agar is used as a nonselective medium to isolate MSSA. MSSA are confirmed by coagulase and cefoxitin testing.

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 will 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 *S. aureus* 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

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.

- 5% Sheep Blood agar (BAP)
- Staph latex reagent or coagulase reagent
- Mueller Hinton agar
- Cefoxitin-30 µg disks

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 cultures for growth consistent with *S. aureus*.
2. Confirm isolates growing on CHROMagar MRSA II with a slide coagulase test.
3. Confirm isolates growing on the BAP with coagulase and cefoxitin disk diffusion.

8.0 Interpretation & Reporting of Results

8.1 MRSA

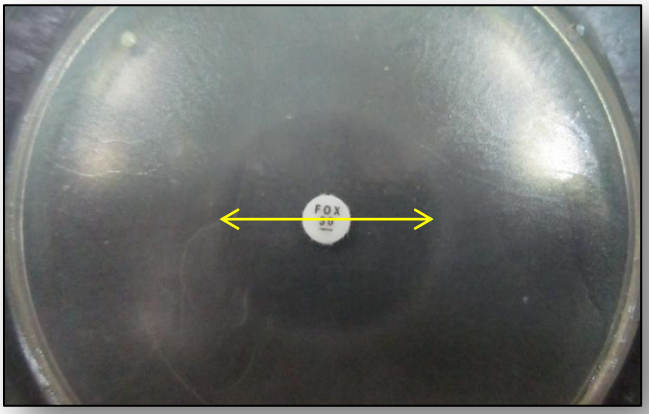
MRSA will grow on both BAP and CHROMagar MRSA II. On CHROMagar, MRSA appear as mauve-colored colonies. If the isolate is confirmed by slide coagulase, report:

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

8.2 MSSA

S. aureus colonies growing on blood agar are medium-sized, cream to yellow, smooth, slightly raised, and hemolytic. The typical appearance of coagulase-negative staph species is nonpigmented, smooth, glistening, and opaque. There are coagulase-negative staph species that may appear hemolytic, including *S. lugdunensis* and *S. haemolyticus*. MSSA colonies will appear on the BAP but fail to grow on the CHROMagar MRSA II medium. Isolates that are coagulase-positive should be confirmed as MSSA by cefoxitin (FOX) disk diffusion. If the isolate is confirmed by slide coagulase and FOX disk diffusion, report:

- **Staphylococcus aureus, NOT MRSA [NOTMR]**



MSSA confirmed by

FOX disk zone of inhibition ≥ 22 mm.

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. MRSA concentrations of lower than 10^6 CFU/mL may yield false negative results on BBL CHROMagar MRSA II.
3. 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.
4. 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.
5. The growth requirements of certain strains of MRSA can lead to their partial or complete inhibition in culture.
6. Incubation in CO_2 is not recommended and may result in false negative cultures.
7. 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

Refer to the MRSA Screen Culture Procedure for CHROMagar MRSA II verification testing.

12.0 References

1. Package insert: BBL™ CHROMagar™ MRSA II, L010089, Rev. 01, September 2010.

13.0 Document Control History

Reviewed by Microbiology Director, Dr. Ann Robinson: 05/06/2014

Reviewed by Microbiology Supervisor, Jerry Claridge: 05/06/2014