Appendix A. Specimen Collection and Screening Protocol

Materials

- Sterile standard culture swabs (BBL CultureSwab™; Becton Dickinson)
- Mannitol Salt Agar (BBL Stacker Plate™; Becton Dickinson)
- HardyCHROM™ MRSA Chromogenic Agar (Hardy Diagnostics)
- Staphyloslide Latex Test (BBL™ Staphyloslide Latex Test for *Staphylococcus aureus*; Becton Dickinson
- Sterile disposable inoculating loops

Procedure

1. Using the sterile culture swab, thoroughly swab a 2" x 2" area of interest. Rotate swab with sampling to expose the entire swab to the sample area.

   NOTE: Do not touch the swab. The swab should not touch an area outside of the 2" x 2" sample area. Swabbing technique should be consistent. Always use aseptic technique.

2. Place the swab back in the transport tube and label with the desired name. Repeat for all areas of interest.
3. Specimen swabs should be plated immediately, or within 24 hours, onto Mannitol Salt agar (MSA). Obtain 1 MSA plate per specimen swab.
4. Using the specimen swab, cover the entire surface of the MSA plate to create a confluent lawn of growth. Alternatively, one may use the swab to perform a standard streak plate on MSA. Properly label the plate. Discard swab. Repeat for all other specimen swabs.

   NOTE: One may choose to perform an enrichment step after swabbing a MSA plate by placing the swab in a 0.5% NaCl broth for 24h followed by an additional MSA inoculation and the subsequent steps 5-11.

5. Incubate for 48-72 hours at 37°C.
6. After incubation, test suspected *S. aureus* colonies from MSA using a *S. aureus* latex agglutination kit, such as the Staphyloslide Latex Test by Becton Dickinson. The latex slide agglutination test is used to differentiate *S. aureus* by detecting Protein A, a clumping factor, from other staphylococci that do not possess the protein. Follow the latex slide test instructions exactly as found in the product insert; there are a variety of commercial latex agglutination kits for confirmation of *S. aureus*
7. Using a sterile inoculating loop, subculture the confirmed *S. aureus* isolate onto HardyCHROM™ MRSA Chromogenic Agar.

   NOTE: Chromogenic agar is light sensitive. Store the plates in the dark at 2-8°C until time of inoculation. Prolonged exposure to light may result in contamination, discoloration, or reduced recovery of the organism.

8. Label plate. Incubate for 48-72 hours at 37°C.

9. Discard all plates with no growth. Parafilm and store all MSA plates with suspected *S. aureus* growth at 2-8°C, if needed for further evaluation.
10. After incubation, MRSA isolates will appear as mauve- colored conies.
11. Record results and discard agar plates.

Additional Information

- All isolates, MRSA or other *Staphylococcus* spp., may also be confirmed by automated testing in a reference laboratory or a hospital clinical laboratory using a Vitek, Microscan, or other commercial automated identification platform. Automated testing will add a higher cost to testing; however, shorter turnaround times on results may be achieved.

- Isolates can be characterized by antibiotic susceptibility testing utilizing a reference laboratory or hospital clinical laboratory. Susceptibility testing identifies which antibiotics the isolate is resistant or susceptible to via incubation with a panel of selected antibiotics (e.g. penicillin, erythromycin, etc.)

- Isolates can also be characterized genetically (e.g. Pulse field gel electrophoresis, DNA sequencing, etc.) by submitting them to a specialty lab (e.g. CDC, state health labs that conduct genetic testing, or other specialty labs). Genetic testing can characterize the isolate as healthcare associated or community associated genotypes.