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# Laboratory Maintenance of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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## Abstract

*Staphylococcus aureus* is an important bacterial pathogen in the hospital and community settings, especially *Staphylococcus aureus* clones that exhibit methicillin-resistance (MRSA). Many strains of *S. aureus* are utilized in the laboratory, underscoring the genetic differences inherent in clinical isolates. *S. aureus* grows quickly at 37°C with aeration in rich media (e.g. BHI) and exhibits a preference for glycolytic carbon sources. Furthermore, *S. aureus* has a gold pigmentation, exhibits β-hemolysis, and is catalase and coagulase positive. The four basic laboratory protocols presented in this unit describe how to culture *S. aureus* on liquid and solid media, how to identify *S. aureus* strains as methicillin resistant, and how to generate a freezer stock of *S. aureus* for long-term storage.

### Keywords

*Staphylococcus aureus*; HA-MRSA; CA-MRSA; growth; strain selection; CDM; freezer stock

## INTRODUCTION

*Staphylococcus aureus* is a gram-positive coccus (~0.6μm in diameter) that colonizes the skin and/or nares of most humans. Given access to other tissues (via tissue disruption or an impaired immune system), *S. aureus* can cause a wide variety of serious clinical manifestations including: pneumonia, osteomyelitis, endocarditis, skin and soft tissue infections (SSTI's), and septicemia. This unit describes methods, reagents, and equipment commonly utilized for the growth, maintenance, and characterization of *S. aureus* in the laboratory. Basic Protocol 1 describes the culturing of *S. aureus* on agar plates, Basic Protocol 2 describes the Oxacillin-salt agar method for determining methicillin-resistance of *S. aureus* strains, Basic Protocol 3 describes the culturing of *S. aureus* in liquid media, and Basic Protocol 4 describes the preparation of a frozen stock of *S. aureus* from a liquid culture. Additional information regarding strain selection (Table 1), unique molecular characteristics of CA-MRSA isolates (Table 2), selective antibiotic concentrations (Table 3), the appearance of cultured *S. aureus* (Figures 1–3), and a recipe for chemically defined media (Table 4) is also provided.

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*CAUTION: Staphylococcus aureus* is a Biosafety Level 2 (BSL-2) pathogen. Follow all appropriate guidelines and regulations for the use and handling of pathogenic microorganisms. No special precautions are necessary when working with methicillin-resistant *Staphylococcus aureus* (MRSA). See *UNIT 1A.1* and other pertinent resources (APPENDIX 1B) for more information.

## Strategic Planning

Unlike other pathogenic bacteria studied in the laboratory, there are many commonly utilized strains of *S. aureus* (Table 1). This is a reflection of the fact that *S. aureus* strains exhibit variation in commonly studied *in vitro* phenotypes, different degrees of virulence in mouse models of infection, and were isolated from a wide variety of infection sites and individuals. As mentioned previously, *S. aureus* is commonly divided into the methicillin-resistant (MRSA) and -susceptible subgroups (MSSA). MRSA strains were first isolated in the 1960's (Jevons, 1961) and it was later determined their resistance was linked to the acquisition of a mobile genetic element (staphylococcal cassette chromosome) harboring the *mec*A gene, (SCC*mec*) (Ubukata et al., 1989). Since the identification of the original SCC*mec* cassette, research on this topic has revealed the existence of other SCC*mec* cassette types that confer additional antibiotic resistance genes to *S. aureus* isolates (for a description of the SCC*mec* types see, (International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009)).

The most commonly studied MSSA isolates include: NCTC8325 (RN1), NCTC8325-4 (RN450), SH1000, RN6390, RN4220, and Newman. NCTC8325 (RN1) was isolated in 1960 from a sepsis patient and was primarily utilized in the laboratory for the propagation of phage 47 and the study of *S. aureus* genetics (NOVICK and RICHMOND, 1965). To make isolation of phage 47 easier, NCTC8325 was cured of three endogenous prophages encoded in its genome via successive rounds of UV mutagenesis, yielding strain NCTC8325-4 (RN450) (Novick, 1967; Herbert et al., 2010). Eventually, it became clear that NCTC8325 exhibited reduced toxin production, pigmentation, and virulence in mice, as compared to other clinical *S. aureus* isolates. Genomic comparison of NCTC8325 to other MSSA strains confirmed that this strain had mutations in *rsb*U (activator of *sig*B) and *tca*R (activator of *spa*) (Herbert et al., 2010), making it a poor model for the study of *S. aureus* genetic regulation and virulence (albeit it has been widely utilized for the study of these two topics). To reverse this loss of virulence, the *rsb*U mutation was repaired in strain NCTC8325-4, yielding strain SH1000 (Horsburgh et al., 2002). Likewise, RN6390 is a more virulent derivative of NCTC8325-4 (produces greater amounts of exoprotein and α-toxin), but it was generated via sequential phage transduction (Peng et al., 1988).

Like SH1000 and RN6390, RN4220 is also a derivative of NCTC8325-4. However, RN4220 was mutagenized via nitroguanoside to enrich for mutants that would efficiently accept plasmid DNA from *E. coli* (Kreiswirth et al., 1983; Herbert et al., 2010). As a result, RN4220 carries 121 SNP's (eleven shared by NCTC8325-4), 4-large scale deletions, an insertion in *agr*A, and a nonsense mutation in *hsd*R (the major restriction endonuclease in *S. aureus*) (Nair et al., 2011) when compared to *S. aureus* NCTC8325. The *agr*A mutation present in RN4220 has been shown to lead to delayed activation of the *agr* quorum sensing

system, which in turn, affects virulence factor production. Thus, RN4220 is not recommended for the study of *S. aureus* virulence regulation or *in vivo* pathogenesis. Instead, RN4220 is used as a cloning intermediate when moving plasmid DNA from *E. coli* into *S. aureus*.

The final MSSA isolate widely utilized in the laboratory is *S. aureus* Newman. Newman is not a derivative of NCTC8325, but was instead isolated from a secondary osteomyelitis infection of a TB patient prior to 1952 (DUTHIE and LORENZ, 1952). Like NCTC8325, and all of its derivatives, Newman has unique features, including: the overproduction of toxins resulting from the constitutive expression of *sae*RS (Adhikari and NOVICK, 2008) as well as mutations in genes encoding for the virulence proteins FnbA and FnbB that limits their surface localization (Grundmeier et al., 2004). Furthermore, the *S. aureus* Newman genome contains four prophages (ΦNM1-4), three of which (ΦNM1, 2, and 4) are known to replicate during *in vitro* culture and animal infections (Bae et al., 2006). Despite these observations, Newman is widely utilized for virulence studies of *S. aureus*.

The most commonly studied MRSA isolates include: COL, Mu50, N315, JH1, JH9, UAMS-1, MRSA252, LAC, SF8300, FPR3757, TCH1516, and MW2 (Table 1). All MRSA isolates are commonly divided into hospital and community acquired strains (HA and CA-MRSA) based on the origin of the disease-causing isolate. Interestingly, this distinction has also come to reflect historical development of *S. aureus* pathogenesis and the emergence of a new MRSA-epidemic. HA-MRSA was first isolated in 1961 and quickly became the dominant hospital acquired infection following the widespread introduction of methicillin treatment in hospitals (Jevons, 1961). The major HA-MRSA strains studied in the laboratory are COL (Sabath et al., 1972; Dyke, 1969), Mu50 (intermediate-vancomycin resistance) (Hiramatsu et al., 1997; Kuroda et al., 2001), N315 (Kuroda et al., 2001), MRSA252 (Holden et al., 2004), UAMS-1 (Cassat, 2006), JH1, and JH9 (Sieradzki et al., 2003). These strains represent the historical evolution of HA-MRSA as well as its geographic diversity, and encompass two main pulse field gel electrophoresis (PFGE) subtypes, USA100 and USA200 (Thurlow et al., 2012). HA-MRSA traditionally causes surgical site infections (SSIs), osteomyelitis, and septicemia in hospital patients.

CA-MRSA, on the other hand, was first isolated in the late 1990's when healthy individuals within the community, who had had no recent contact with a hospital setting, contracted fatal *S. aureus* infections (CA-MRSA) (Thurlow et al., 2012). It was later determined that these isolates were distinct from their HA-MRSA counterparts in that they mostly cause skin and soft tissue infections (SSTI's), occasionally cause necrotizing pneumonia, and can readily infect healthy individuals (Millar et al., 2007). The most commonly utilized CA-MRSA strains studied in the laboratory are SF8300 (Charlebois et al., 2002), LAC (Kennedy et al., 2008), FPR3757 (Diep et al., 2006), TCH1516 (Gonzalez, 2005), and MW2 (Baba et al., 2002). SF8300, LAC, FPR3757, and TCH1516 all belong to the dominant CA-MRSA PFGE subtype, USA300, while MW2 belongs to the second most common CA-MRSA PFGE subtype, USA400 (Thurlow et al., 2012). Additional CA-MRSA lineages are new recognized in the literature, but laboratory work characterizing these strains has only recently been undertaken (e.g. ST239).

While the variability in pathogenesis between HA and CA-MRSA isolates implies detectable genetic differences between these two MRSA sub-groups, CA-MRSA isolates exhibit a large amount of heterogeneity world-wide, making it difficult to suggest any single molecular test for identification of CA-MRSA isolates. Furthermore, CA-MRSA strains have begun to replace HA-MRSA strains in many hospitals around the world, confounding the traditional classification of MRSA isolates into the HA and CA categories (Popovich et al., 2008; Jenkins et al., 2009; Moore et al., 2009; Hultén et al., 2010). Thus, MRSA isolates have more recently been categorized into HA and CA sub-categories based on sequence typing (i.e. phylogeny) and pulse-field gel electrophoresis, of digested genomic DNA (Enright et al., 2000; McDougal et al., 2003). However, some simple molecular characteristics do separate CA-MRSA isolates from their HA-MRSA counterparts, albeit these characteristics are not shared by every CA-MRSA strain (Table 2) (Thurlow et al., 2012; Li et al., 2012).

## Basic Protocol 1: GROWTH OF *S.AUREUS* ON SOLID MEDIA

*S. aureus* forms small, shiny, gold colonies on most solid media after 1 day of growth at 37°C. The size and color of the colonies is media and strain dependent (media containing low levels of glycolytic carbon sources will produce colonies that are less pigmented as are some strains harboring specific mutations that affect pigmentation, Figure 1). Several different media have been described for growing *S. aureus* including: Brain Heart Infusion (BHI) Agar, Tryptic Soy Agar (TSA), Todd Hewitt Agar (THA), Luria-Bertani (LB) Agar, Mueller-Hinton Agar (MHA), and Blood Agar. BHI, TSA, THA, MHA, LB Agar are all rich media and will allow for robust growth of *S. aureus*. Blood agar is also a rich media but allows for the additional observation of hemolysis. Most strains of *S. aureus* are β-hemolytic (i.e. produce complete lysis of red blood cells resulting in a zone of clearing, Figure 2) as a result of alpha toxin production. *S. aureus* colonies grown on blood agar are small, shiny, pigmented (to varying degrees depending on the glucose content of the blood agar used and the strain of *S. aureus*), and surrounded by a zone of clearing. Antibiotics can be added to the media as necessary according to the concentrations outlined in Table 3. It should be noted that USA300 isolates contain plasmids carrying an erythromycin and tetracycline resistance cassettes and therefore genetic manipulation of USA300 involving antibiotic selection is restricted.

### Materials

*S.aureus* frozen stock (Basic Protocol 3)

BHI Agar plates

Sterile wooden applicators

37°C incubator

1. Using a sterile wooden applicator streak out a small amount of *S. aureus* from the frozen stock onto a small section of a BHI plate (~1/4) using aseptic technique.

   Only a small amount of frozen stock is needed, enough to be visible at the end of the stick.

2. With a new sterile wooden applicator, streak out a new quadrant of *S. aureus* by passing through the initial quadrant several times. Repeat this process 1–2 more times, passing a new applicator through the most recently streaked quadrant.

3. Incubate the plates for 16–24 hrs at 37°C.

## Basic Protocol 2: TESTING *S. AUREUS* FOR METHICILLIN RESISTANCE

As stated in Strategic Planning, *S. aureus* resistance to penicillinase-stable penicillins (i.e. methicillin, oxacillin, nafcilin, etc.) is encoded by the *mec*A gene located on the SCC*mec* cassette (Ubukata et al., 1989). The most accurate method for identifying methicillin-resistant *Staphylococcus aureus* (MRSA) is to test for the presence of the *mec*A gene by PCR (Unal et al., 1992). However, phenotypic confirmation of methicillin resistance is highly recommended regardless of the outcome of the PCR reaction as strains carrying the *mec*A gene can exhibit susceptibility to methicillin (Araj et al., 1999; Bignardi et al., 1996). The primary methods for demonstrating *S. aureus* methicillin resistance are the cefoxitin disk screen test, the latex agglutination test for PBP2a (the enzyme encoded by *mec*A), and the Oxacillin-salt agar screen (Sakoulas et al., 2001). While the latex agglutination test for PBP2a has been shown to be the most accurate method for determining *S. aureus* resistance to methicillin, the Oxacillin-salt agar screen is the simplest method and is still very accurate (99% sensitivity and 98.1% specificity) (Sakoulas et al., 2001). According to the Clinical and Laboratory Standards Institute (CLSI) *S. aureus* strains exhibiting growth on MHA supplemented with 2% NaCl and ≥ 4 μg/ml Oxacillin are considered methicillin-resistant while *S. aureus* strains only exhibiting growth on MHA supplemented with 2% NaCl when Oxacillin-levels are ≤ 2 μg/ml are considered methicillin-susceptible. Additional information regarding the assay described below can be found in the Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement published by the CLSI (document M100-S17).

### Materials

*S.aureus* streak plate (Basic Protocol 1)

Mueller-Hinton Agar (MHA) plate supplemented with 2% NaCl and Oxacillin (2 and 4μg/ml)

0.5 McFarland Standard

Sterile Microfuge tubes

Plastic Cuvettes

Vortexer

Spectrophotometer

35°C incubator

1. Using aseptic technique, transfer several well isolated colonies of *S. aureus* from the streak plate (plate prepared in Basic Protocol 1) into 1ml of PBS, contained in a 1.5 ml microfuge tube, by rubbing the inoculating loop against the side of the tube at the liquid-air interface.

There should be enough bacteria to be visible on the loop.

2. Thoroughly resuspend the transferred *S. aureus* by vortexing the mixture or pipetting up and down repeatedly.

3. Adjust the turbidity of the *S. aureus* suspension to that of a 0.5 McFarland Standard using a spectrophotometer.

   McFarland Standards are a turbidity reference for the dilution of bacterial suspension into a range of desired concentrations. McFarland Standards can be prepared by mixing barium chloride with sulfuric acid (McFarland Standard 0.5: 0.05ml 1% barium chloride with 9.95ml 1% sulfuric acid) or from purchased suspensions of latex particles (greater accuracy and longer shelf life). The S. aureus suspension should be diluted with sterile PBS to achieve an absorbance equal to the 0.5 McFarland Standard using a wavelength of 600 or 625nm.

4. Aliquot 10µl of the diluted *S. aureus* onto the surface of a MHA-salt plate, a MHA-salt plate with 2µg/ml Oxacillin, a MHA-salt plate with 4µg/ml Oxacillin.

5. Allow spots to dry and then incubate the plates at 35°C for 24 hrs.

6. Methicillin-resistant *Staphylococcus aureus* (MRSA) will exhibit growth on all three plates while methicillin-susceptible *Staphylococcus aureus* (MSSA) will grow on all plates except the MHA-salt plate with 4 µg/ml Oxacillin.

## Basic Protocol 3: GROWTH OF *S. AUREUS* IN LIQUID MEDIA

*S. aureus* grows rapidly in broth culture at 37°C with aeration. As such, overnight cultures of *S. aureus* are typically started at the end of the day just prior to leaving the lab using either an isolated colony from an agar plate (Basic Protocol 1), a second broth culture, or a frozen stock (Basic Protocol 4). Several different media have been described for growing *S. aureus* in broth culture, including: Brain Heart Infusion (BHI), Tryptic Soy Broth (TSB), Todd Hewitt Broth (THB), Luria-Bertani (LB) Broth, and Chemically Defined Media (CDM). There is no specific advantage or disadvantage to using BHI, TSB, THB, or LB; all are rich media and will allow for *S. aureus* to grow to a high concentration overnight. CDM, on the other hand, is generally utilized for growth experiments involving the limitation or addition of specific nutrients. Regardless of the media, it should be noted that *S. aureus* is auxotrophic for Arginine (Emmett and Kloos, 1979) and Proline (Li et al., 2010), that *S. aureus* cannot use inorganic sulfur sources (*e.g.* sulfate or sulfite) (Soutourina et al., 2009), and that *S. aureus* exhibits a preference for glycolytic carbon sources during aerobic growth (Seidl et al., 2009). Repeated passaging of *S. aureus* in liquid media can result in the accumulation of mutations and thus, should be avoided. Overnight cultures of *S. aureus* will exhibit gold pigmentation, although the extent of pigmentation is media and strain dependent (Figure 3).

### Materials

BHI broth

Streak plate of *S. aureus*

Wire inoculating loop

Sterile 15–20ml culture tubes (with caps)

37°C Incubated shaker

1. Using aseptic technique, transfer a single colony of *S. aureus* from the streak plate (plate prepared in Basic Protocol 1) into the aliquoted broth by tilting the culture tube and rubbing the inoculating loop against the side of the tube at the liquid-air interface.

   There should be enough bacteria to be visible on the loop.

2. Grow cultures overnight (~16–18hrs) at 37°C with shaking (250rpm).

   S. aureus is a facultative anaerobe. Thus, the overnight culture does not have to be shaken at 250 rpm, however the growth rate and maximum bacterial density achieved may be affected. To maximize aeration, place the tube on the shaker at an angle. Following overnight growth, the culture will be in stationary phase.

## Basic Protocol 4: PREPARATION OF *S. AUREUS* FROZEN STOCKS

Long-term storage of *S. aureus* should occur at −80°C to prevent accumulation of mutations. To prepare a frozen stock of *S. aureus* add an aliquot of an overnight culture (Basic Protocol 3) to sterile DMSO as described below. The addition of DMSO prevents complete freezing of the cells and thus limits cellular damage as a result of the transition to −80°C. Sterile glycerol (final concentration 25–50% once mixed with bacteria) is frequently used as an alternative to DMSO. There is no distinct advantage to using one agent over the other except that DMSO thaws more slowly than glycerol, and thus maintains a low culture temperature for a longer period of time if freezer failure should occur.

### Materials

Overnight culture of *S. aureus* (see Basic Protocol 3)

Sterile DMSO

Sterile 2ml cryotubes

−80°C Freezer

1. Add 150µl of sterile DMSO to one sterile cryotube using aseptic technique.
2. Add 850µl of overnight culture to the same cryotube using sterile technique.
3. Vortex or invert briefly to mix.
4. Store cryotube at −80°C.

## REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see **APPENDIX 2A**; for suppliers, see **SUPPLIERS APPENDIX**.

### Brain Heart Infusion (BHI) Agar Plates

Add 52g of BD BBL™ Brain Heart Infusion Agar to distilled water in a 2L flask. Alternatively, 40g of BBL™ Tryptic Soy Agar or BBL™ Luria-Bertani Agar can be similarly utilized. Add a stir bar to the flask and then place the flask on a stir plate to mix. Bring volume up to 1L using distilled water. Loosely cover the top of the flask with aluminum foil and then autoclave the media on liquid cycle (use 30 minute cycle to prevent glucose carmelization). Once sterilized, place the flask on a stir plate and stir the media at a low setting for ~45 minutes or until the media has cooled to 65°C. Add antibiotics as necessary and then pour the agar into sterile petri plates (~20–25ml/plate). Allow agar to solidify at room temperature for at least 1 day (minimizes condensation) then store the plates, top down, in plastic bags at 4°C.

### Brain Heart Infusion (BHI) Broth

Add 37g of BD BBL™ Brain Heart Infusion to distilled water in a 2L flask. Alternatively, 30g of BBL™ Tryptic Soy Agar, 25g of BBL™ Luria-Bertani Agar or 30 g of BBL™ Todd-Hewitt Broth can be similarly utilized. Add a stir bar to the bottle and place the bottle on a stir plate to mix. Bring volume up to 1L using distilled water. Once the powder is completely dissolved, remove the stir bar, loosely cap the bottle, and autoclave the media on liquid cycle (do not tightly screw on the bottle cap or else the bottle will become highly pressurized in the autoclave; use 30 minute cycle to prevent glucose carmelization). Once sterilized, the broth may be stored at room temperature or 4°C.

### Mueller-Hinton Agar Plates Supplemented with 2% NaCl and Oxacillin (MHA-salt plates)

Add 38g of BD BBL™ Mueller-Hinton II Agar and 20g NaCl to distilled water in a 1L bottle. Add a stir bar to the flask and then place the flask on a stir plate to mix. Bring volume up to 1L using distilled water. Loosely cover the top of the flask with aluminum foil and then autoclave the media on liquid cycle (use 30 minute cycle). Once sterilized, place the flask on a stir plate and stir the media at a low setting for ~45 minutes or until the media has cooled to 65°C. Add antibiotics as necessary (i.e. 2μg/ml and 4μg/ml Oxacillin) and then pour the agar into sterile petri plates (~20–25ml/plate). Allow agar to solidify at room temperature for at least 1 day (minimizes condensation) then store the plates, top down, in plastic bags at 4°C.

### Chemically Defined Media (CDM)

Make the solutions outlined in Table 4 and sterilize as indicated (the amino acids and bases are made and stored separately to maximize the longevity of storage). Once cooled, all solutions should be stored at 4°C. Place a stir bar into a 250ml beaker and place the beaker onto a stir plate. Add 10mls of the salt solution, 1ml of each amino acid, 1ml of each base, 0.1ml of the vitamin solution, and 0.1ml of the trace elements solution to the beaker while slowly stirring the mixture. Add carbon sources as desired and then adjust the pH of the media to 7.4 using 10M NaOH. Bring the final volume of the media up to 100ml using distilled water and then filter sterilize the media.

### STERILE DMSO

Sterilize 100% DMSO using a syringe filter (0.2μm Nylon syringe filter attached to a 10ml syringe). Do not attempt to sterilize the DMSO using a nitrocellulose filter because it will dissolve once it is exposed to the solvent.

## COMMENTARY

### Background Information

*S. aureus* was first isolated from a knee abscess in 1881 (Ogston, 1881). Since then, *S. aureus* has become the most dominant nosocomial infection in the developed world (i.e. the MRSA epidemic). Although the prevalence of HA-MRSA amongst hospital-acquired infections is currently waning, new subsets of MRSA (USA300, USA400, ST239, etc.) have arisen within the community setting (Climo, 2009). These community-acquired MRSA (CA-MRSA) isolates exhibit increased capacity to cause disease in healthy individuals (Weber, 2005). The primary clinical presentation of USA300 is skin and soft-tissue infections, but it has also been found to cause fatal necrotizing pneumonia (Millar et al., 2007).

MRSA as a whole is capable of causing a wide variety of clinical presentations, including: pneumonia, surgical site infections, endocarditis, myocarditis, osteomyelitis, toxic shock syndrome, scalded skin syndrome, and septicemia. The ability of *S. aureus* to cause such varied diseases is largely attributed to the diversity of virulence factors encoded in the *S. aureus* genome and the innate resistance of *S. aureus* to nearly all facets of the human immune system. One such virulence factor produced by *S. aureus* is the distinct yellowish-orange pigment called staphyloxanthin (*aureus* means “golden” in Latin). Pigment production has been shown to aid in the resistance of *S. aureus* to oxidative damage (Katzif et al., 2005; Pelz et al., 2005; Liu, 2005). Other major virulence factors encoded by *S. aureus* include: toxins (*e.g.hla*, *hld*, *hlg*, *set8*, *psm*α), proteases (*e.g. spl*, *sspC*, *aur*), lipases (*lip* and *geh*), adhesins (*e.g. cflA*, *cflB*, *fnbA*, *fnbB*), coagulases (*coa* and *vwb*), and factors involved in immune resistance (*e.g. ldh*1, *hmp*, *katA*, *spa*, *eap*, *chp*, *sak*, and capsule) (Foster, 2005; Foster and Höök, 1998; Dinges et al., 2000).

Aside from these obvious virulence factors, *S. aureus* is also a fast growing organism that is capable of utilizing a variety of carbon sources (interestingly, USA300 isolates grow faster *in vitro* than their HAMRSA counterparts, (Thurlow et al., 2012)). As mentioned earlier, all *S. aureus* strains exhibit an *in vitro* auxotrophy for arginine and proline, although for proline, this is only during growth on glycolytic carbon sources (*e.g.* glucose). This is caused by catabolite-mediated repression of the proline biosynthetic genes via the catabolite response regulator, CcpA (Li et al., 2010). Thus, careful consideration of the growth media chosen for experimentation should be taken, given that *S. aureus* exhibits extensive metabolic regulation.

### Critical Parameters and Troubleshooting

Proper growth and maintenance of *S. aureus* in the laboratory is not difficult. Inocula need not be large in order to generate robust growth, and chances for contamination are rare given the short incubation times/fast growth rate of *S. aureus* cultures. Furthermore, contaminants

are easily discernible from *S. aureus* by several easily testable phenotypes, all of which S. aureus is generally positive for, including: gold pigmentation, catalase activity, coagulase activity, β-hemolysis, and growth on Mannitol salt agar. Repeated passage of *S. aureus* in the laboratory is not recommended as it may result in the accumulation of undesired mutations.

### Anticipated Results

For growth on BHI agar, *S. aureus* should appear as small, shiny, gold colonies after 12–24 hrs of growth at 37°C. Likewise, overnight cultures of *S. aureus* in BHI broth should appear golden and highly turbid.

### Time Considerations

*S. aureus* is a fast growing microorganism. Liquid cultures generally need only 12–24hrs to achieve a high density while most strains of *S. aureus* will become visible as colonies on a plate after 12–36hrs of growth at 37°C.

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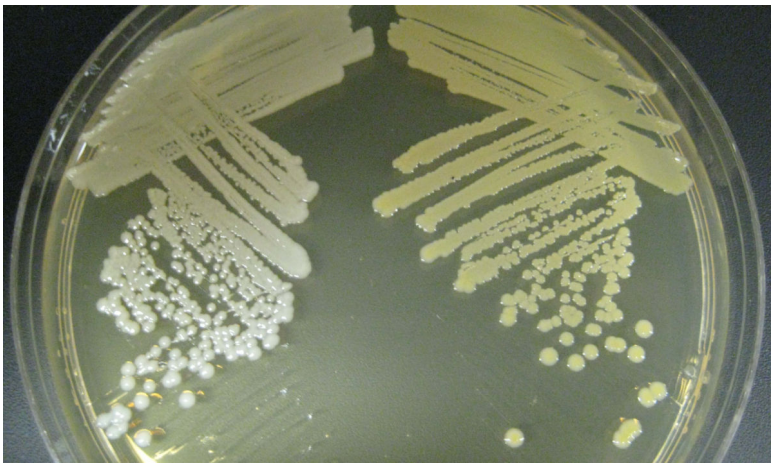

**Figure 1.**
*Staphylococcus aureus* strains SF8300 (right) and RN4220 (left) grown on Brain Heart Infusion agar for 18 hr at 37°C.

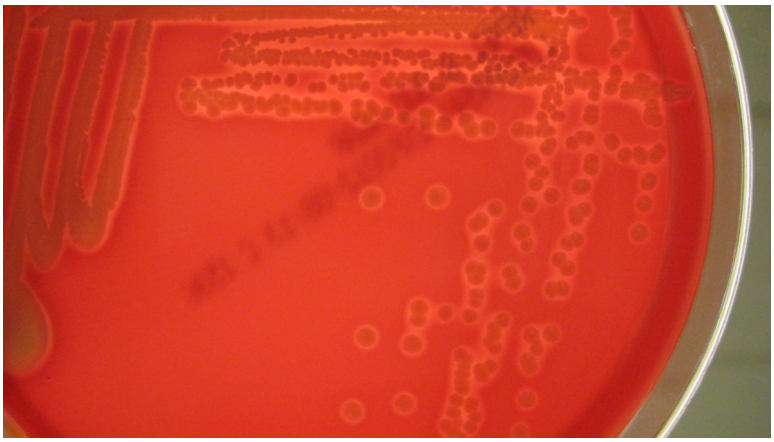

**Figure 2.**
*Staphylococcus aureus* strain SF8300 grown on Tryptic Soy Agar with Sheep's Blood for 18 hr at 37°C.

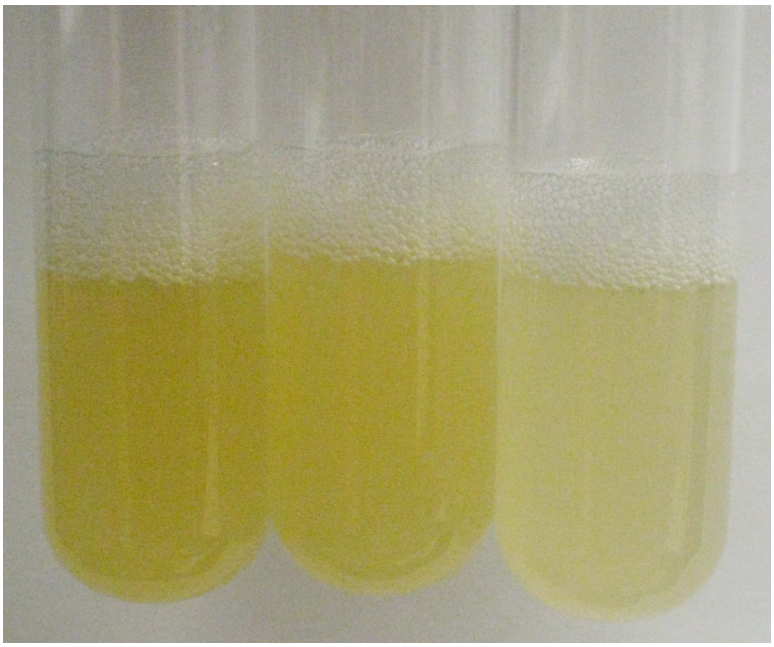

**Figure 3.**
Growth of *Staphylococcus aureus* (SF8300, left; COL, middle) and *E.coli* (DH10B, right), in Brain Heart Infusion Broth for 18 hr at 37°C with aeration.

**Table 1**

Commonly Utilized *Stophylococcus* aureus Strains

| Strain | Classification | PFGE Type | Sequenced | Origin |
|---|---|---|---|---|
| NCTC8325 (RN1) | HA-MSSA | N/A | Yes | Corneal ulcer isolate from sepsis patient (1960). |
| NCTC8325-4 (RN450) | MSSA | N/A | Yes | Laboratory strain, derived from NCTC8325 by UV mutagenesis. |
| SH1000 | MSSA | N/A | Yes | Laboratory strain, derived from NCTC8325-4 by complementation of *rsb*U |
| RN6390 | MSSA | N/A | No | Laboratory strain, derived from NCTC8325-4. |
| RN4220 | MSSA | N/A | Yes | Laboratory strain, derived from NTC8325-4 using chemical mutagenesis (MNNG). |
| Newman | HA-MSSA | N/A | Yes | Osteomyelitis Isolate, TB patient (pre-1952) |
| COL | HA-MRSA | N/A | Yes | Clinical isolate, Colindale, England (1961) |
| Mu50 | HA-MRSA | USA100 | Yes | Vancomycin resistant SSI isolate from male infant, Japan (1996) |
| N315 | HA-MRSA | USA100 | Yes | Pharyngeal smear isolate, Japan (1982) |
| JH1 and JH9 | HA-MRSA | USA100 | Yes | Sequential blood isolates, vancomycin resistant infective endocarditis in patient with underlying congenital heart disease, Baltimore, USA (2003) |
| UAMS-1 | HA-MRSA | USA200 | No | Osteomyelitis Isolate, McClellan Veterans Hospital, Little Rock, Arkansas, USA (1995) |
| MRSA252 | HA-MRSA | USA200 | Yes | 64-year-old female, fatal post-op septicemia, United Kingdom (1997) |
| LAC | CA-MRSA | USA300 | No | SSTI Isolate, County Jail, Los Angeles, USA (2002) |
| SF8300 | CA-MRSA | USA300 | No | Wound Isolate, San Francisco, USA (1996–2003) |
| FPR3757 | CA-MRSA | USA300 | Yes | Wrist abscess isolate, 36-year old HIV-positive white male, San Francisco, California USA |
| TCH1516 | CA-MRSA | USA300 | Yes | Clinical isolate, septic arthritis, 14-year-old black male, Texas Children's Hospital, Houston, Texas, USA (2002) |
| MW2 | CA-MRSA | USA400 | Yes | Blood isolate, fatal septicemia, 16-month-old American Indian female, North Dakota, USA (1998) |

**Table 2**

Unique CA-MRSA Genes/Attributes

| Characteristic | Description | Exceptions |
|---|---|---|
| ACME Cassette | Arginine Catabolic Mobile Element carried by most USA300 CA-MRSA isolates(contains 33 genes, including *speG* and a fully functional *arc* operon) | USA300 clones in S. America |
| *speG* | Encodes a spermine acetyltransferase on the ACME island. Provides spermine resistance. | See Above |
| *lukSF-PV* | Encodes for Panton-Valentine leukocidin or PVL (pore forming toxin). | ST72 in Asia |
| *sasX* | Encodes for surface anchored virulence factor involved in aggregation and attachment to host cells. | Non-ST239 isolates |

**Table 3**

Antibiotic Stock Solutions for Use with *S. aureus* Cultures

| Antibiotics | Solvent | Stock Concentration | Working Concentration |
|---|---|---|---|
| Chloramphenicol | EtOH | 20mg/ml | 20 μg/ml |
| Erythromycin | EtOH | 5 mg/ml | 5 μg/ml |
| Spectinomycin | $H_2O$ | 100 mg/ml | 100 μg/ml |
| Kanamycin | $H_2O$ | 50 mg/ml | 50 μg/ml |

**Table 4**

Chemically Defined Media for Growth of *S. aureus* Cultures.

| Solution(s) | Item | Amount (g) | Volume (ml) | Solvent | Sterilize |
|---|---|---|---|---|---|
| Salt Solution | $K_2HPO_4$ | 70 | 1000 | $H_2O$ | Autoclave |
| | $KH_2PO_4$ | 20 | | | |
| | $(NH_4)_2SO_4$ | 10 | | | |
| | $MgSO_4 \uparrow 7H_2O$ | 25.6 | | | |
| Amino Acids | Phe | 1 | 250 | 1M $NH_4OH$ | Autoclave |
| | Iso | 7.50E-01 | 250 | 1M $NH_4OH$ | Autoclave |
| | Tyr | 1.25 | 250 | 1 N HCl | Autoclave |
| | Cys★ | 5.00E-01 | 250 | 1 N HCl | Autoclave |
| | Glu | 2.5 | 250 | 1 N HCl | Autoclave |
| | Lys | 2.50E-01 | 250 | 1 N HCl | Autoclave |
| | Met | 1.75 | 250 | 1 N HCl | Autoclave |
| | His | 7.50E-01 | 250 | 1 N HCl | Autoclave |
| | Trp | 2.50E-01 | 250 | 1 N HCl | Autoclave |
| | Leu | 2.25 | 250 | 1 N HCl | Autoclave |
| | Asp | 2.25 | 250 | 1 N HCl | Autoclave |
| | Arg | 1.75 | 250 | 1 N HCl | Autoclave |
| | Ser | 7.50E-01 | 250 | $dH_2O$ | Autoclave |
| | Ala | 1.5 | 250 | $dH_2O$ | Autoclave |
| | Thr | 7.50E-01 | 250 | $dH_2O$ | Autoclave |
| | Gly | 1.25 | 250 | $dH_2O$ | Autoclave |
| | Val | 2 | 250 | $dH_2O$ | Autoclave |
| | Pro | 2.50E-01 | 250 | $dH_2O$ | Autoclave |
| Bases★★ | Adenine | 1.25E-01 | 250 | $dH_2O$ | Autoclave |
| | Cytosine | 1.25E-01 | 250 | $dH_2O$ | Autoclave |
| | Guanine | 1.25E-01 | 250 | $dH_2O$ | Autoclave |
| | Thymine | 5.00E-01 | 250 | $dH_2O$ | Autoclave |
| | Uracil | 1.25E-01 | 250 | $dH_2O$ | Autoclave |
| Vitamin Solution | Thiamine | 1.00E-01 | 100 | $H_2O$ | Autoclave |
| | Niacin | 1.20E-01 | | | |
| | Biotin | 5.00E-04 | | | |
| | Ca Pantothenate | 2.50E-02 | | | |
| Trace Elements | $FeCl_3$ | 8.00E-01 | 100 | H2O | Filter Sterilize |
| | ZnCl | 6.95E-03 | | | |
| | MnCl ↑ 4H20 | 9.90E-03 | | | |
| | Boric Acid | 6.00E-04 | | | |

| Solution(s) | Item | Amount (g) | Volume (ml) | Solvent | Sterilize |
|---|---|---|---|---|---|
| | CoCl2 ⬆ 6H20 | 3.97E-02 | | | |
| | CuCl2 ⬆ 2H20 | 2.56E-04 | | | |
| | NiCl2 ⬆ 6H20 | 2.38E-03 | | | |
| | Na2MoO4 ⬆ 2H20 | 3.58E-03 | | | |

★Cystine not cysteine

★★May need to heat or titrate using KOH to get into solution