**Isolate ID Protocol**

**RECORD:** Pigment, hemolysis, and catalase results on isolate sheet.

**INOCULATE x2:** Pass to trypticase soy broth

**GRAM STAIN**

**INCUBATE:** 37°C overnight (~18 hours)

**COAGULASE TEST** Gram positive, catalase positive cocci and record results on isolate sheet

**STORE**

**FOR PERMANENT STORAGE**

(2) twist-top tubes

(2) 900uL inoculated TSB + 900ul TSB with 30% glycerol

**CHECK**: Streak for isolation to check for purity

1 tube frozen to be sent to MSU (-80 degrees C)

1 tube frozen at UVM (-80 degrees C)

**PLATE**

Blood Agar

Follow quarter milk plating procedure

**INCUBATE:** 37°C for 48 hours

**RECORD:** Take notes on quarter milk sample recording sheet

Pick isolates from plates

**STORE**: Keep extra milk

Pass on to blood agar until isolated

Begin ID process

**Quarter Milk**

**Bulk Tank Milk (BTM)- *picking Staph/Strep organisms***

**PLATE**: 100ul

Chromagar MRSA (1)

Chromagar SA

Edwards

Blood agar

U

Strep selective

Mannitol salt

U

U

U

U

1:10

U

**INCUBATE**: 37°C for 24 hours

Count # MRSA colonies (rose/mauve) out of total # colonies on Chromagar MRSA

Count # S. aureus colonies (pink/mauve) out of total # colonies on Chromagar SA

**INCUBATE**: 37°C for 48 hours

Pick isolates from plates

Pass on to blood agar until isolated in pure culture

**KEY**

Begin “Isolate ID protocol”

**BA: Blood Agar**

**MSA: Mannitol Salt**

**CA SA: Chromagar S. aureus**

**CA MRSA: Chromagar MRSA II**

**U: Undiluted**

**Bulk Tank Milk (BTM)- *Endogenous inhibitor plates***

**En**

**PLATE**: 100ul

Chromagar SA

Blood agar

U

U

1:10

**INCUBATE**: 37°C for 24 hours

Flip plate, streak with Staph

**INCUBATE**: 37°C for 24 hours

Pick isolates, pass to blood agar

**INCUBATE**: 37°C for 48 hours

Check for purity, re-plate if necessary onto BA and incubate 48 hours

Once pure isolate, begin “Isolate ID protocol”