Microbiology Lab Experiment Changes

Experiment #: 5-20, 5-21

Title: Microbial Flora of Throat and Skin / Staph. Identification

Live Organisms: normal flora, *S. aureus, S. epidermidis, S. saprophyticus, Streptococcus sp.*

Changes:

Procedure (work individually)

1. You don't need to wear gloves because you are swabbing yourself and we don't have any tongue depressors.

2. We are using blood agar for throat and mannitol salt agar for skin.

3. Streak for isolation on the blood plate. Swab the mannitol salt agar plate. Note: you should wet the swab with sterile saline prior to rubbing swab vigorously on skin.

Next Lab Period

4. Next lab period you will determine the type of hemolysis on your BAP.

5. Also next lab period: You will identify the organisms from your mannitol salt agar plate.

To further differentiate *S. aureus* from *S. epidermidis* you may inoculate a coagulase tube.

- Each student should inoculate a coagulase tube using a "golden" colony from the MSA plate. If you have no golden colonies, then use a white colony.
- If you have no significant growth on MSA, then inoculate a coagulase tube with the *S.* aureus or S*. epidermidis* cultures provided.

To differentiate *S. epidermidis* from *S. saprophyticus*, you may swab the entire surface of a TSA plate with the suspect organism from a white colony on the MSA plate and then apply a novobiocin antibiotic disc.

6. Observe the demonstration plates of beta-hemolytic *Strep. pyogenes* as well as mannitol plates with *Staph.* Record the type of hemolysis observed on your blood agar plate and perform a gram stain on a colony (hemolytic if possible).

Take Home Lesson: If given a blood agar plate, you should be able to recognize the different types of hemolysis (alpha, beta, gamma). If given a mannitol salt agar plate or a coagulase tube inoculated with *Staphylococcus*, you should be able to differentiate *S. aureus* from *S. epidermidis*. You should refresh your knowledge of the purpose of mannitol salt agar.

Test	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Staphylococcus saprophyticus***
Mannitol Fermentation	**+, yellow agar, growth**	**-, no yellow agar, growth**	**-, no yellow agar, growth**
Coagulase	**+, clot forms**	**-, no clot**	**- no, clot**
Novobiocin Sensitivity	**Susceptible (>=22mm zone)**	**Susceptible (>=22mm zone)**	**Resistant (<=17mm zone)**