Selective and differential media assignment

Lab Session 8: Selective and Differential Media, Unknown ID (Enzyme Based Tests) Oct. 25-31st (To be turned in PRIOR to start of recitation for lab 8) Name: _____ _____ Objective: Analyze microbes from last week. Understand the use of antibiotics on microorganisms. Gain more knowledge about selective media and differential media. Practice use of the catalase test, coagulase and the oxidase test. Observe microbial flora of the nose. Significance: Understand the use of Mannitol salt agar, blood agar and MacConkey agar plates which must be used based on the components of the bacteria.

The catalase test will be used to understand the difference in facultative anaerobic and groam positive from aero tolerant anaerobes. The coagulase test converts fibrogen to fibrin, causing clotting, and is used to prevent phagocytosis of material. The oxidase test can be used to distinguish bacteria: positive organisms and negative organisms dependent upon the color obtained in the test. In the Nasal swab, we will learn to isolate particular flora. Procedure: Throat Microbial Flora 1. Analyze the flora from last week and gram stain an isolated colony. . Receive TA approval of the gram stain. Procedure: Disinfectant and Antiseptics 1. Use a ruler to measure the diameter of the zones of inhibition (mm) 2. Record each antibiotic zone size in the chart, determine if it is sensitive, intermediate, or resistant; and report to TA. Procedure: Selective and Differential Media 1. Label the MacConkey agar plates. Streak the plates for the following organisms: Enterobacter aerogenes, Salmonella enterica, and Staphylococcus aureus, and sterilize the loop after streaking each quadrant. . Label a sheep blood agar plate and use a loopful of Enterococcus faecalis to inoculate it. Streak

the plate. 3. Repeat step 2 for Streptococcus pyogenes 4. Transfer an A disc with flamed forceps to the cross of first and second quadrant of the Streptococcus pyogenes. Press disc into agar gently. Cover and invert plate and sterilize forceps. 5. Repeat step 2 for Streptococcus pneumonia. 6. Repeat step 4 with a P disc onto the Streptococcus pneumonia plate. 7.

Obtain a loopful of Streptococcus pyogenes and streak onto a bile esculin slant 8. Repeat step 7 with a loopful of Enterococcus faecalis. Put all of the tubes in the incubation rack. Make sure each plate is placed in incubation. Procedure: Catalase Test 1. Obtain bacterial growth from the inoculated slant and use a sterile loop to put on slide. Add one drop of hydrogen peroxide to the smear. Determine if the organism is catalase or catalase-negative based. Discard the slide. Procedure: Coagulase test 1. Put a drop of plasma on a glass slide.

Obtain a loopful of Staphylococcus aureus from the slant and mix well using the inoculating loop on the slide. Repeat the process using Staphylococcus epidermidis. Note findings and discard slide. Procedure: oxidase test 1. Obtain filter paper and put in Petri dish. Then put 3-5 drops of oxidase reagent on a paper. Remove organisms from the TSA slant with a wooden applicator stick. Rub the bacteria in the reagent. Note the colored obtained within 5-10 seconds. 2. Discard Filter paper and Petri dish in red biohazard bag.

Put wooden applicators in beaker of disinfectant. 3. Note findings. Procedure: Flora of Nose 1. Do not write anything on mannitol salt agar plate. 2. Use aseptic technique as obtain cotton swab and put it 2-3 cm into one nostril.

Swab the inner nostril. 3. Procede to roll the swab over one quadrant of the plate. Discard the swab in the BacDown? beaker. 4. Streak the plate with sterile loop. Do NOT sterilize the loop after each quadrant. 5. Rubber band the plate, invert it, and place in incubation.