**Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

**NORMAL FLORA AND OPPORTUNISTIC PATHOGENS**

**Blood agar plates** contain a typical nutrient growth medium enriched with 5% sheep blood.  It is useful for encouraging growth of **fastidious organisms**.  It also can show results of **hemolytic enzymes** produced by some organisms.  Remember from the selective and differential media lab, that alpha hemolysis is the incomplete breakdown of the blood cells leading to a green coloration around the colonies, beta hemolysis is the complete breakdown of RBCs leading to a clear area around the cells, and that gamma hemolysis is growth on the media with no breakdown of the blood cells and no change to the media.

**Mannitol salt agar** is a **selective medium** for isolation of pathogenic staphylococci.  Growth of most bacteria other than staphylococci (**halophilic**) is inhibited by the high concentration of salt in the medium.  Pathogenic staphylococci fermenting in the mannitol sugar turns the phenol red indicator bright yellow.  Non-pathogenic staphylococci produce small colonies surrounded by red or purple zones.

PREPARATION AND MATERIALS

Provide a TSA 5% Sheep Blood agar plate and a mannitol salt agar plate for each student, as well as sterile cotton swabs and sterile water.

**Procedure:**

**Part I: Throat Biota**

Have a partner take a throat culture with a sterile cotton swab.  The swab must go to the back of the pharynx quickly, not touching the tongue or the salivary secretions.  Rub gently and withdraw.  The gag reflex is expected.  Streak the sample for isolation on blood agar.  The instructor will demonstrate the simple but effective “**candle-jar**” incubation technique which provides a higher CO2 concentration and less oxygen (compatible with the pharynx flora requirements).  Observe after 48-hour incubation and do a Gram stain on the selected colonies.

Typical flora of the pharynx is alpha-hemolytic *Streptococcus viridans* which grows best in greater than normal CO2.  A few class members may represent those individuals which are beta-hemolytic *Streptococcus pyogenes* carriers.  They do not show inflammation, but harbor this true pathogen.  *S. pyogenes* is often implicated in infectious pharyngitis (“strep throat”).

**Part II: Skin & Nasal Biota**

Use a grease pencil to mark a mannitol salt plate into two sections.  With a cotton swab moistened in sterile water, sample the skin of the forearm, and streak on one-half of the plate.  (Be sure to label correctly).  With another swab, take a sample from the nasal cavity to streak on the other area of the dish.  Incubate 48 hours, observe and Gram stain selected colonies.

Most people have *Stapylococcus epidermidis* as normal flora on skin and in the nasal cavity.  Some people harbor *Staphylococcus aureus* in these areas.  Strains of *S. aureus* can be responsible for various disease patterns, from purulent skin lesions to exotoxic food poisoning.

NOTE:  In a heath care career, a worker is often asked to take samples (specimen) from a patient and prepare them for transport to the laboratory for diagnostic testing.  It is critical that the specimen be taken and treated appropriately so that the lab work will be valid.  Much emphasis will be placed on such techniques in the clinical courses of the health care curriculum.

**Part III: Urine Cultures**

The number of microorganisms that are present in a  properly collected urine sample may indicate the presence of a urinary tract infection.  Urine is typically sterile as it exits the bladder.  However, as it passes through the urethra and out of the body, it flushes out microorganisms and becomes contaminated with the normal microbiota of the genitourinary system.  The majority of UTI's are caused by invasion of the normal flora by way of the urethra.

**Materials:**

* Calibrated 1uL loops
* TSA with 5% blood agar and EMB agar plates
* Urine collection containers

**Procedure:**

Collect a clean catch, midstream sample of urine in the container provided.

Using the disposable 0.001 calibrated loop, inoculate  each plate with a straight line of urine down the center of the plate.

Carefully spread the urine over the surface of the plates with your metal inoculating loop using a zig-zag pattern.

Incubate for 48 hours.

Count the number of colonies on the plate.  Multiply by 1,000 to convert to numbers per milliliter.  Report in colony-forming units/ml or CFU's/ml

       < 10,000 CFU =  no work up unless it is a specimen from surgery or a catherized specimen.

        > 10,000 CFU buy < 100,000 CFU =  work up organisms with susceptibility if a gram  
         negative rod.    (possible contamination or inadequately refrigerated)

      > 100,000 CFU = indicates significant bacteria.  Work up all organisms with susceptibility.

If 3 or more different colony types on a plate then report as contaminated.

If there is a predominate organism present even if less than 100,000, then usually it will be worked up and a MIC reported.

Main organisms of clinical significance found:  E. coli (the most common cause of UTI), Proteus spp., Pseudomonas, Enterobacter, Citrobacter, Klebsiella, Candida albicans, Streptococcus, and Enterococcus faecalis.

**Part IV: Mouth Biota/Susceptibility Test**

Provide a tube of Snyder Test Agar for each student that has been boiled for 10 minutes then held in a water bath at 45o C.  Block of paraffin/or sugarfree gum for each student.  Sterile container for saliva.  Sterile 1 ml pipette for each student.

The human mouth provides a warm, moist environment for many species of organisms.  Within your mouth lives a tremendous amount of microorganisms, including fungi, protozoa, viruses and bacteria.

Dental caries or cavities are caused by bacteria and saliva is known to contain over a million bacteria per milliliter.  All cavities begin with the formation of plaque.  Plaque is a gummy substance on the surface of the enamel that is a mixture of various bacteria and the end products of carbohydrate hydrolysis and fermentation.  The production of acids by these bacteria causes tooth decay.  At a pH of 5.5 or lower, demineralization of the tough, protective enamel begins.

*Streptococcus* and lactobacilli are two of the biggest acid producers and it now seems that *Streptococcus mutans* seems to be the most important one and is usually the one that initiates tooth decay.

In this exercise you are estimating your susceptibility to dental caries by measuring the rate at which your own oral lactobacilli generate acid.

The low pH and reduced environment provided in Snyder Test Agar inhibits most oral flora.  Lactobacilli thrive, producing acid from the medium’s dextrose.  If your lactobacilli drop the pH of the medium below about 4.6, the bromcresol green indicator changes from green to yellow.  This color change shows that your mouth biota produce enough acid to decalcify teeth procedure.

**Procedure:**

1. Obtain a tube of liquified Snyder Test Agar that has been boiled for 10 minutes and then cooled to 45\* C.
2. Obtain a piece of sugarfree gum or paraffin and chew for 3 minutes without swallowing.  Collect your saliva in a sterile container.  Chewing removes the bacteria from your teeth.
3. Shake your saliva to resuspend the microorganisms.
4. With a 1ml pipette, transfer 0.2 ml of saliva to tube of test agar.
5. Mix the contents of the tube by rotating the tube vigorously between the palms of the hands.
6. Incubate the tube at 37\* C.  Examine the tube at 24-hour intervals to see if the bromcresol green indicator has changed to yellow.  If the medium turns yellow in 24-48 hours the individual is said to susceptible to caries.