LAB LECTURE NOTES FOR WEEK 6

REGARDING SMEARS, GRAM STAINS AND MICROSCOPY

1. Graded gram stain unknowns will be handed back today **or** as soon as possible.

   a. We are primarily interested in **gram reaction** and **shape**. (Remember that "morphology" is just another word for shape.) If the cells are cocci, **then** some special terminology for cell arrangement can apply (streptococcus, staphylococcus, tetrad, etc.) which can best be discerned if you have a **very thin smear** with the cells spread out as much as possible.

   b. We are just interested in the unknown on page 13 (not the known cultures), and it should not be identified to any species. Theoretically we could have given out hundreds of different species that look identical to each other with the gram stain. How to begin differentiating them beyond the gram stain is the theme of Experiment 7.

2. **Smear-making hints:**

   a. Hold the slide well above the flame (at least a foot) if you need to have the smears **dry** a bit faster; this is best accomplished by holding the slide with the **hand**. **Then**, be sure to use the **metal forceps** to **heat-fix** the slide by drawing it through the flame (smear-side up) three times without stopping and over-heating the slide.

   b. Always remember that when it comes to making, heat-fixing, staining and observing the slides, **you can stop at any point in the procedure and pick it up again later** – even weeks later – and the smears will not have deteriorated in the interim period. We won't keep repeating this every period. (It is believable!)

3. **Microscopy Hints:**

   a. When preparing smears, have the slide facing up with the etched-in letters readable as the word: G L O B E. Then you know which side is indeed up throughout the staining and observing processes, and you can have the letters on the left side for convenience in orienting the smears.

   b. As it should be obvious that these etched letters are on the **same plane** (the same level) as your cells, you could start focusing with the 10X lens on any of the letters. Then, with the slide perfectly level on the stage, you can move the slide across the stage and find each smear in focus.

   c. When observing your slides under the microscope, make sure that the **bottom surface is cleaned off** of any residual stain and other material that could accidently become focused upon with the 10X objective. It's also a good idea to **clean off the condenser lens**, as the 10X objective can also focus on that as well. **We only want to focus on what is on the top surface of the slide which is where the smear is**. Otherwise the oil immersion lens – when you attempt to swing it into place – will only jam into the slide and be useless. This fact should be obvious. The slides are not paper-thin, and even if they were, there is still some distance to cover with the microscope objective from one surface to the other.

   d. Only clean off the 10X objective lens with **oil-free lens paper**!

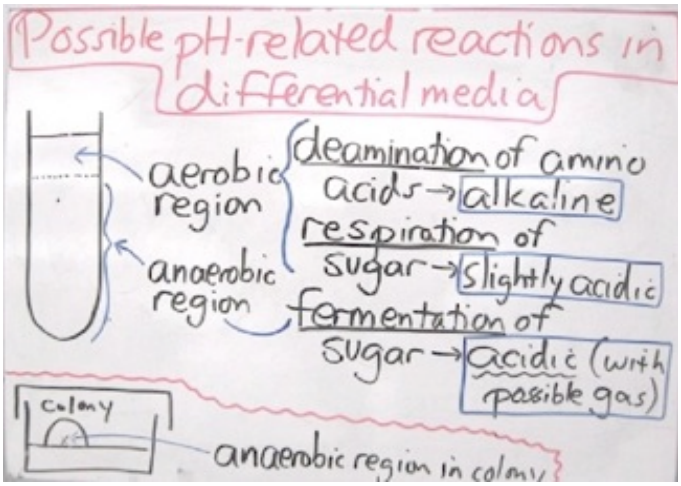
VIRTUAL EXPERIMENTS 5A AND 5B

1. **Oxygen Relationships:** Note handout passed out last week regarding catabolism. As you follow through Virtual Experiment 5A, note the summary table on page 24.

2. **Siderophores:** Helpful diagram is presented **on today's handout** and is also reproduced on-line. (See "siderophore" in the links box.) A siderophore is an example of an organic compound that most organisms can produce in order to allow transport of inorganic iron compounds into the cell. Those that do not produce a siderophore therefore need it provided to them, and according to the definitions in Appendix D, a siderophore would therefore be considered a **growth factor** for such organisms. And **iron is an example of a trace element** as you also see discussed in Appendix D.

EXPERIMENT 7A – PERIOD 1

1. In Experiment 7A, we will study how **comparative physiology and morphology** can help to characterize different kinds of bacteria. And we can touch on how organisms can be characterized by their DNA.
   a. **Phenotypic** tests have been the major method by which bacterial species have been defined in the 1900's. New methods which compare the **genotypes** (DNA & RNA) of known and unknown organisms are now being used in identification and also the establishment of new species, but – like most labs – we do not have the facilities to do this type of work routinely, so we are sticking with "classical," phenotypic tests in Microbiology 102. This is gone over in the introduction to Exp. 7.
   b. On occasion a well-known species may have to be split into two or more species on the basis of genotypic tests, and also two or more species have been merged into one when it was found out their DNAs were practically identical. Then, new phenotypic may have to be found whose results will correlate with how the species are genetically defined.
2. Next week, we will be observing "positive" and "negative" reactions in various, carefully-formulated differential media and learn what this might mean regarding the physiology of particular organisms.
   a. We are doing more with differential media which are interpreted by pH reactions, having dealt with MacConkey Agar in Exp. 4 (see diagram in the lab lecture notes for Period 4). Recall from our discussion that most of our bacteria which grow on our organic media tend to produce an **<u>alkaline reaction</u>** due to the release of **ammonium** from the aerobic deamination of amino acids. We can formulate a medium which includes a particular sugar (such as glucose or lactose), and fermentation of that sugar by a particular organism results in an **<u>acidic reaction</u>** which will overneutralize the alkaline reaction. This is how we will test for glucose and lactose fermentation in Exp. 7A.



   b. We also work with other differential media which do not depend on pH changes to interpret their reactions such as motility medium, nitrate broth, tryptone broth and starch agar.
3. The introduction to the concepts of **anaerobic respiration** and **extracellular enzymes** are also important parts of this experiment and are best gone over next time.
4. So, in Experiment 7, we get a little practice in the **identification of unknowns** which utilizes the data-base of the results we find for the 12 known cultures. Everyone should find identification of their unknown to be easy **if aseptic technique is utilized and only <u>pure cultures</u> are tested**!

EXPERIMENT 5C – PERIOD 2

1. Each pair will get a short form on which you can record the absorbance reading and CFUs/ml for your particular time point. (Hopefully you had recorded the number on the tube which is the time point!) Remember that we always intend to calculate the **number of CFUs per gram or ml of the <u>undiluted</u> sample**, and we started making dilutions from the undiluted sample (rather than from a 1/10 dilution as we did in the hamburger experiment). Also, as we had inoculated one ml from the dilutions into the plates, the "**plated dilution**" of each plate corresponds to the dilution it was inoculated from. For example, plating one ml from a 10^{-5} dilution of the original, undiluted culture will result in a "plated dilution" of 10^{-5}. Continuing with the same example, the **dilution factor** (which you see on the short form as having a positive exponent) will therefore be 10^{5}.
2. **Averaged** CFU/ml and absorbance for each time point will be **provided next period** such that we can go ahead according to "For Your Assignment" on page 24B.