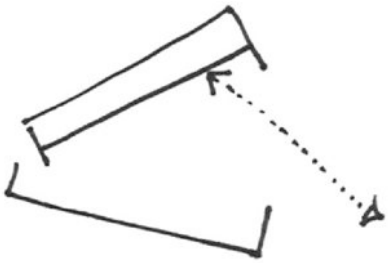
LAB LECTURE NOTES FOR WEEK 4

EXPERIMENT 1 – PERIOD 3

1. Proceed according to manual. Note colonies on top of and within the medium in the plates. Those embedded in the medium often have a lens-shaped appearance. **Colonies are always studied thru the top** of the plate, **but it is easier to count them thru the bottom** as indicated in the procedure. (The **grid** mentioned in the manual is just a random grid for convenience in counting.) Only if the colonies appear uniformly dispersed would we count a half or quarter of the plate and then estimate from that the total number of colonies on the plate. Remember how to hold and observe plate if moisture on the top lid is obscuring vision. ——>



2. Recall that you were given **dilutions** of soil and lake water with which to inoculate the petri plates. In the case of our soil and water samples, if you had inoculated the same amount of **undiluted** material, there would be an overgrowth of colonies on the plate. Theoretically, we could plate smaller and smaller amounts of the undiluted material, but we don't have the facilities to dispense amounts much less than 0.1 ml, and the sample we take would be less representative. So, we do the **equivalent** thing by plating out dilutions.

3. Instructors must always be careful how to approach bacterial quantitation. The following way of figuring out CFUs per ml or gram of sample in Experiment 1 is simple and can be put as follows on the board with an appropriate diagram:

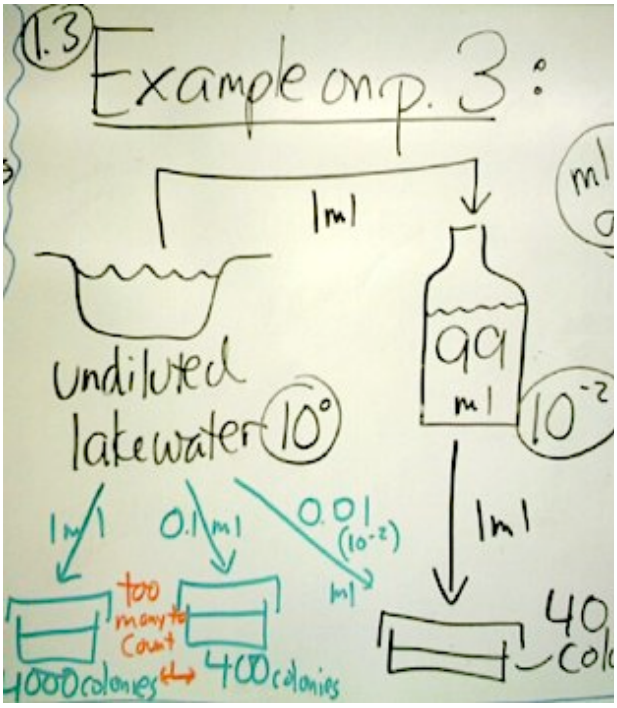
> *If* **40 colonies** arise from having plated **1 ml** of a **1/100 dilution** of lake water,
> *then* there would have been (40 X 100=) **4000 CFUs** per **1 ml** of the **undiluted** lake water.

Also:

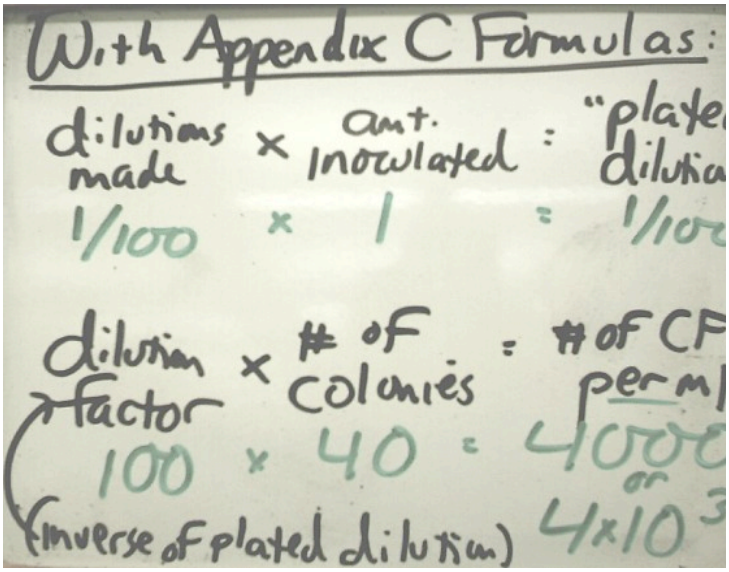
> *If* **40 colonies** arise from having plated **1 ml** of a **1/10,000 dilution** of soil,
> *then* there would have been (40 X 10,000=) **400,000 CFUs** per **1 gram** of the **undiluted** soil.

We should convert to scientific notation. **Note the "equivalence" of grams and milliliters**. (We don't use the term "microliters" (μl) unless we find it is absolutely necessary to do so – such as in connection with an explanation of pipettor designations. One thousand microliters make up a milliliter.)

4. A diagram of the procedure for the **lake water sample** is shown on the right. Different lake water samples tend to vary considerably from each other, but we try to go to a moderately contaminated lake and usually find that a 1/100 dilution is fine for our purposes.
   a. When the 10^{-2} (1/100) dilution is made, one ml of the undiluted lake water sample is spread out into a total volume of 100 ml.
   b. Note that 1 ml of a 10^{-2} dilution is equivalent to plating 10^{-2} ml of the original sample; we label the plate 10^{-2} as that is not only the "final/plated dilution" according to our formulas but it also represents that part of the original sample that is plated.
   c. Also note that 1 ml of the undiluted (and even 0.1 ml) will give us too many colonies to count in this example, and 0.01 ml is too small an amount for our equipment – so we do the next best thing and that is to make our plate from a dilution.



5. How to use the Appendix C formulas to figure out the lake water example is shown on the right.



6. So, to estimate the number of colony-forming units (CFUs) per gram or ml of a sample, one needs to know **just these three things**:
   - **Dilution of the sample** (e.g., 1/10,000 for the soil sample; 1/100 for the H_2O sample) –>
   - **Amount** of the dilution which was **inoculated into the plate** (1 ml)
   - **Number of colonies** on the plate after incubation.

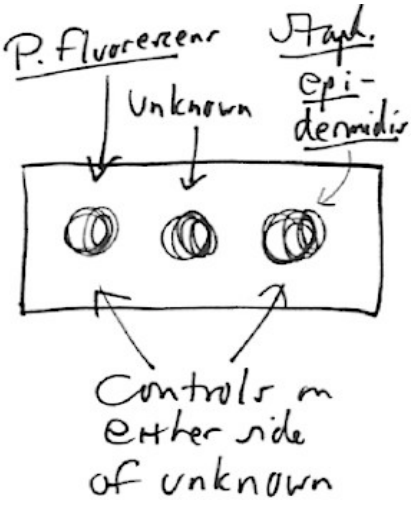
EXPERIMENT 2 – PERIOD 2

1. Procedure today is self-explanatory. Growth of a population of bacteria in a broth medium (that is, (a liquid medium) is indicated by turbidity (cloudiness). Usually the bacterial growth has settled out during the week's incubation. Careful mixing or vortexing will suspend the cells and you will see a cloudy culture.

PRIORITY 2 TODAY –> EXPERIMENT 3A – PERIOD 3 – GRAM STAIN ONLY

1. **MAKING SMEARS FROM CULTURES GROWN ON A SOLID MEDIUM:** When using cultures growing on solid media, one has to take cells from the surface growth and immerse them in a drop of water to start with. Heavy smears must be avoided, so just a **tiny amount** of cells is taken with the **needle**. What ultimately results (when the slide is stained and observed) is usually a "cleaner" preparation than what one gets with liquid cultures. If the smear is too heavy, always move to the edge where the cells are well-separated and their shapes and staining reactions can be observed properly.

2. **GOOD THINGS TO KNOW ABOUT MAKING SMEARS:**
   a. **Clean slides:** One must be sure to use **clean slides**. Note again fig. B2 on **page 91** regarding handling the tube and loop. Put all three organisms on one slide and **remember to record the number of the unknown**.
   b. **Drying the smears:** (Not mentioned in the lab manual.) If you need to dry your smears more quickly and efficiently, **hold the slide by hand at least a foot above the flame**. If you feel your hand getting too warm, raise it higher. We can't heat the smears too much while drying – hence the use of the hand **rather than the forceps**!
   c. **Heat-fixing:** Here is where we **use the forceps** to hold the slide which is passed through the upper part of the flame three times as demonstrated by the instructor.



3. **OBSERVATIONS: Be sure to observe the known cultures first** to see if the gram stain procedure was correctly done. We are only indicating **gram reaction and shape** of the unknown (and not the known cultures). **See p. 122** for terminology associated with **cell shape**; arrangement isn't as important as basic shape. Don't try to **identify** the organism, as many different species can look identical under the microscope, and we may have given you an unknown that is neither of the control cultures. We will be learning about other tests used to differentiate species of bacteria.

WITH PLENTY OF THINGS TO DO THIS WEEK THAT ARE OF HIGHER PRIORITY, THE **ENDOSPORE STAIN (on the slide saved from last time) WILL BE PUT OFF UNTIL NEXT WEEK**.

PRIORITY ONE TODAY –> EXPERIMENT 4A – PERIOD 1

1. In this experiment, we are building on what we have already learned about "**dilution theory**" in Experiment 1. The overall explanation of the concept is given in **Appendix C**. However, an **easier** explanation is on the web as pointed out earlier. (See the box on our main website page.)
2. Note the overall setup of the procedure summarized in the box on page 18. We are starting with one ml of a 10^{-1} dilution of hamburger and making successive 1/10 dilutions. The dilutions are cumulative such that we will ultimately be making a 10^{-5} dilution of the hamburger (equivalent to one part hamburger in one million parts total volume). Note demonstration of the Vortex mixer.
3. When we inoculate 0.1 ml into a plate rather than 1.0 ml, we expect the inoculum to soak into the medium where a 1 ml amount would probably take all period and then some to do so. To use an example: A **0.1 ml** inoculation from a **10^{-4}** dilution is equivalent to making a **1 ml** inoculation from a **10^{-5}** dilution (you probably made similar adjustments in dealing with solutions in Chemistry), and we then label the plate 10^{-5}.
4. Getting to the procedure: As hamburger slurries are hard to pipette, **we have passed them out already in a <u>one ml</u> amount**. When we make our first dilution beyond that (a 1/10 dilution of the original 1/10 dilution – that is, a 10^{-2} dilution of the hamburger), it is actually best to add the 9 ml dilution blank to the tube containing the 1 ml of hamburger slurry.
5. Demonstrate the **use of the pipettors <u>following page 95</u>**. (Note: Usually everyone has some familiarity with these devices which is definitely a good thing.) We have P200's (with yellow tips) to dispense 0.1 ml amounts and P1000's (with blue tips) to dispense 1.0 ml. How to adjust volumes is shown on page 95.
6. Demonstrate the **spread-plating technique** – **<u>also on page 95</u>** – noting the **potential hazard of flaming ethanol**.
   a. Simply dip the hockey stick into the ethanol and **<u>touch</u>** it to the flame of the bunsen burner and let the ethanol burn off. This procedure may be repeated again. **Do not hold the hockey stick in the flame; that will bet it too hot.**
   b. As indicated in the directions, spreading the 0.1 ml inoculum on the plates can begin with the "most dilute" plate and proceed to the "most concentrated," without having to re-flame the hockey stick! It's OK to switch from one medium to the other in the process.
7. As for the two plating media that we use in this experiment, they are part of the discussion in Appendix D. Terms such as "all-purpose," "selective" and "differential" refer to how media are classified. *WE WILL GO OVER THIS NEXT TIME.*
   a. **Plate Count Agar (PCA)** is classified as an **all-purpose** medium. It is designed to support the growth of commonly-found, easily-grown, chemoheterotrophic bacteria – the major type of organism that we study in Microbiology 102. As mentioned before, chemo-heterotrophic bacteria are (1) good for explaining various life processes in all types of bacteria (and higher forms of life) and (2) include important pathogens and food spoilage organisms (which can grow easily in us and in food).
   b. **MacConkey Agar (MAC)** is classified as a **selective-differential** medium. It **inhibits gram-positive organisms** (therefore **selecting** for gram-negatives) and it also **differenti-ates** between colonies of organisms according to **whether or not they ferment lactose**. More about this later.

FOR NEXT TIME: Be sure to have read over <u>Appendix D</u> and also <u>Exp. 5C</u> (especially the introduction and the aseptic technique precautions). Re-reading **<u>Appendix C</u>** will make a lot more sense now. Work on the <u>practice</u> dilution problems which are indicated in the manual (page 19) as **<u>Virtual Experiment 4B</u>**; the link is on our website. These are practice problems (not to be handed in) and there is a link to the solutions. We will pass out the actual take-home problem set soon. (Also keep up with the website updates.)