LAB LECTURE NOTES FOR WEEK 3

(Things mentioned below that are not gone over in lab will hopefully be reviewed next week.)

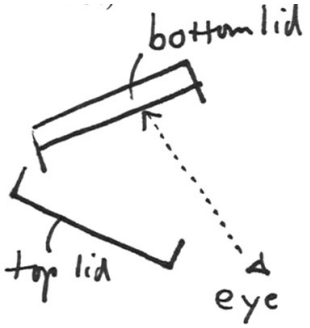
GENERAL INTRODUCTORY COMMENTS

1. Always best to get cultures out from the incubator before the lab lecture starts.
2. **Clean-up procedures:** These include wiping the desk area with paper towel soaked in disinfectant **before** as well as after work. Also where to discard slides, tubes and plates.
3. **Saving slides:** We don't have slide boxes. **However, you can save slides in an empty petri dish** (available on center table or cart) and **store them in any of the side drawers that do not have a colored tag on them**.
4. **Microscopes** (specifically the ones in the cabinet which we use for stained slides):
   a. Our microscope handout (which is now inserted into the manual as pages 90A and 90B) is meant to be useful; if not, please let us know! It is important to use the **iris diaphragm** to regulate the light and not the condenser.
   b. Appendix A in the manual can be consulted regarding the theory and use of the microscope, but some of the directions may not be applicable to the scopes we use.
5. Always **check the schedule** to see what is coming up in the next week. We won't be always reminding you about that each time. Also **check the website updates**.

EXPERIMENT 1 – PERIOD 2

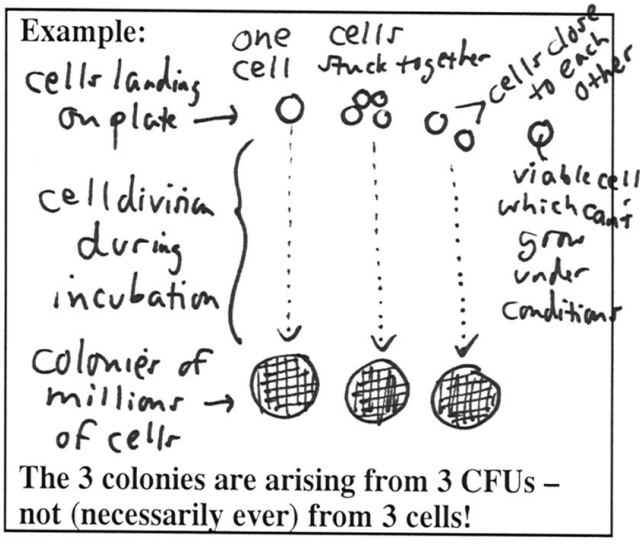
(Introduction and procedure hopefully read over for today; ditto for Exps. 2 and 3.)

1. Observing plates made last time: (Note: In the summer session, the plates probably need extra incubation, so it may be best to look at them after the weekend.) As a general rule, always look at colonies from above them – not through the bottom of the plate (that is, through the medium)! We incubate our plates upside down to prevent any water condensed on the top lid from dripping down on the colonies, causing them to run together. (The condensed water isn't itself contaminated.) If moisture is in the way of viewing colonies through the top lid, hold the plate above the head, cracking the plate a little and observing thru the opening, such that contamination from above is decreased. **Standard operating procedure is shown on the right. –>**



2. The best demonstrations of bacterial colony diversity are shown by your plates and those of your neighbors. Do check them out. Remember **not to open plates that have mold colonies**.

3. Re-emphasize terms given in introduction about cells, colony-forming units (CFUs) and colonies – and how to use the terms correctly!
   a. **CFU** is simply one or more cells which are placed on a petri plate which give rise to the formation of a colony through cell division.
   b **Colony** is simply a visible mass of millions of cells that arose from a CFU. The actual number of viable cells in a sample is always greater than – never less than – the number of CFUs in the sample.



4. We do not say"young and old cells" when we are really talking about the cells in young and old cultures. A colony can be any number of days old, and one which has incubated for several days would certainly be called "old." However an "old cell" could be just 20-30 minutes old since it pinched off from its sibling, and it could be ready to divide into two new cells. Note comments

about this in the introduction to Experiment 1. This will be important when we get to gram-staining where it will become obvious that we always should be working with "young cultures." (Note the use of an "old" *Bacillus cereus* culture and a "young" one.)

5. **Habitats** of microorganisms. That which is found dropping in from the air should not implicate the air as a habitat, as microorganisms do not metabolize and multiply in the air.

6. Note that procedure says Experiment 2 on staining is a higher priority item today, so we should observe the plates we made last time as our **last** item of business today. Note that **Step 4 is optional** – that is, to do as time permits.

7. What we should do first today is Step 1 where we inoculate some more plates – this time using the "pour plate" technique which will lead to a rough quantitation of microorganisms in a certain amount of a sample. More about quantitation next time.

8. How to pour a plate aseptically is shown on page 96; **instructor must demonstrate**. Generally, one bottle of melted medium is used to pour four plates.

9. After plates solidify, they should be incubated upside down (medium side up). As we do not meet often – and the directions occasionally mention incubating for a certain number of days – we can turn the incubators into refrigerators (around 5°C) which will **slow down or stop growth so the plates won't overgrow or dry out**.

EXPERIMENT 2 – PERIOD 1

1. Note the discussion of **aseptic technique** in the introduction. To help achieve aseptic technique, we must always wipe the work area before and after lab with disinfectant. Before work, be sure to wipe the entire area free of dust, including the area near the center of the table.

2. Step 1 of the procedure: (May need to clarify what is to be done with the tubes.) Referring to **page 91**, demonstrate **tube-to-tube transfer**. Especially if the tubes are plugged, then a brief flaming is necessary of the open ends of the tube. If tubes are capped, then flaming is optional.

3. Step 3 of the procedure: Referring to **pages 93-94**, demonstrate **how to streak a plate**. The main purpose is to get **isolated colonies** by initially **spreading out the CFUs**. Need to be able to start with a mixed culture and get individual, isolated colonies of each component of the mixed culture. **Emphasize the 3-phase method** on page 94 when starting out. Must be sure to **flame the loop** between the "phases." **DO NOT HOLD THE TUBE AND PLATE SIMULTANEOUSLY.**

4. Hopefully you saw the videos about tube-to-tube transfers and streak-plating. You can also get to them (and more!) by going to the **YouTube** site and then to the "**johntubeseven**" channel.

EXPERIMENT 3 – PERIOD 2

1. In preparation of bacterial smears (eventually to be stained), **the importance of clean slides** needs to be emphasized. Slides right out of a new box should be clean enough. If it ever appears that drops of liquid will bead up and not spread out, the slide definitely needs some cleaning such as a good rubbing with a paper towel. Eventually when the slide is observed with the microscope, there must never be material accumulated on the bottom surface of the slide; this can interfere with viewing and focusing!

2. For the slides to be made in Experiment 3 today, all three cultures need to be put on each slide as shown in the manual Instructor can demonstrate and include a quick review of heat-fixing. Note diagram in the Appendix (**page 91** – fig. B2) of holding the tube and loop. For aseptic technique, we cannot let organisms from the environment contaminate our culture, and we cannot let the organisms being studied contaminate ourselves and the environment. Light smears are the standard; smears should be barely visible when dry. When all 3 smears are on the same slide (with the controls on either end of the slide as shown in the manual), we will be able to judge the success of the tricky Gram stain procedure we will be performing.

3. Some general things about staining (can go thru this if time permits or just refer to manual):

   a. Bacterial cells are nearly transparent when unstained, and it is quite difficult to discern details of unstained cells. Staining enhances contrast – allowing observation of bacterial shape and sometimes internal structures.

   b. In water environment around pH 7 (neutrality), most bacterial cells carry a net negative charge. Most bacteriological dyes contain a color-emitting molecule (chromophore) which is positive in charge; these dyes are the basic dyes. As opposite charges attract, the basic dyes tend to stain the bacterial cells. (There are also acidic dyes which contain negatively-charged chromophores. These dyes tend to stain the background rather than the cell.)

   c. **Simple stains** simply color bacteria with a single basic/positive dye – such as the crystal violet stain we did last time.

   d. **Differential stains** involve procedures that enable the bacteriologist to discern special structures or differences between certain types of cells. A brief summary is given on page 145. The most classic example of a differential stain is the **gram stain**.

4. **The gram stain is explained on page 117**: Important things to consider are in the top half of the page – especially the need for **young cultures** and **thin smears**! (The Experiments refer to items in the Appendix from time to time; don't forget to return to the experiment after using the appendix!)

   a. Use of gram stain: To classify bacteria into two groups based on structure of **cell wall** (which, with the cell membrane, constitutes the **cell envelope**). This is generally the first step in the identification of an unknown bacterium. **Most** bacteria have **either** a gram-positive type of cell wall or a gram-negative type. (Diagram can be put on board.) The **thicker** peptidoglycan layer of the **gram-positive** cell wall correlates with a **relative resistance of the cell to decolorizing agents**.

   b. Discovered by Christian Gram in 1884 who was actually trying to develop a special staining method for eucaryotic cells. (An accidental discovery, figured out decades later.)

   c. **Procedure** in bottom half of page 117. Note where it says to just drain off the water; slide does not have to be completely dry before the next step. Trickiest part is with the alcohol-acetone: The slide is held lengthwise and alcohol-acetone is applied at one end, and it should evenly cover the slide as it flows downward. **No more than 10 seconds of continuous application is enough**; then the slide is carefully rinsed under the tap water. **By the way, too much water will decolorize!** Note that a **counterstain is defined as a stain which stains cells which have been decolorized** in a differential staining procedure; it is meant to impart a distinctly different color from the primary stain.

5. The *Escherichia coli* and young *Bacillus cereus* cultures should give the two opposite staining reactions typical for these organisms. As for morphology (**shape**), see the diagram on **page 122**. We usually see definite rods or cocci, but sometimes we observe oval-shaped cells that make us wonder if they're cocci or rods. This is explained on p. 122.

FOR THE NEXT TIME WE MEET (Week 4):

See the schedule for what is coming up which should always be a general rule. This includes the items in the **Virtual Experiments** and **Items Due** columns. Also, we will be using the **pipettors**. for the first time in lab, and this may be your first time using a pipettor in association with aseptic technique. Pipettor operation is summarized on **page 95**.

Always check the homepage of the website for updates. A recent update links to things about the virtual experiments.