LAB LECTURE NOTES FOR PERIOD 4

EXPERIMENT 3 – PERIOD 3
(CATCHING UP ON THE ENDOSPORE STAIN)

1. Essential theory is given on page 118 along with the procedure.
2. About cell division:
   a. Bacteria generally reproduce by binary fission where a vegetative cell divides into two separate vegetative cells **when nutrients are available**.
   b. There are some organisms (notably species of the genera *Bacillus* and *Clostridium*) that undergo another kind of division **when they sense that nutrients are running out**. In this case, the vegetative cell divides into two – but instead of separating, one of the cells (the endospore) is actually formed <u>inside</u> the other cell. Therefore, as the enclosing cell and the endospore are actually siblings, it is a misnomer to call the enclosing cell the mother cell.
3. The endospore is a highly resistant type of cell and can survive for very long periods and then germinate into a regular vegetative cell when nutrients and proper growing environment are again available. Endospores will probably be discussed in some detail in the lecture course. We learn more about endospores in Experiment 11C where we actually isolate endosporeforming bacteria from soil.
4. The steps of the endospore stain are somewhat analogous to those of the gram stain in that there is a primary stain (malachite green), a fixer (the heat treatment that drives the stain into the endospores), a decolorizing agent (water, which decolorizes the vegetative cells), and a counterstain (safranin, which stains the decolorized vegetative cells red). End result: Endospores are green and vegetative cells are red.

EXPERIMENT 4 – PERIOD 2
(WITH REFERENCES TO APPENDICES C AND D)

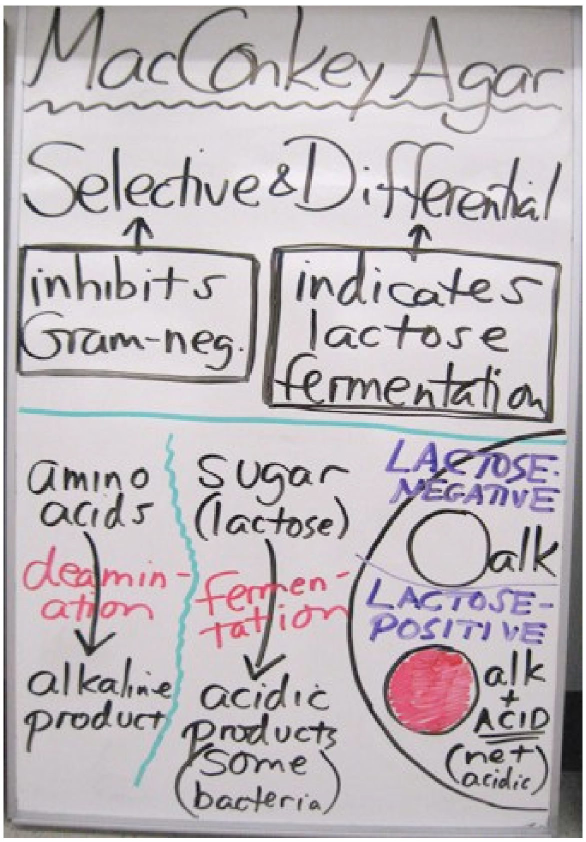
1. Any problems yet regarding **dilution theory** (Appendix C)? Having done the practice problems will make the take-home dilution problem set a breeze.
2. **Appendix <u>D</u> is filled with good basic stuff relating to bacterial physiology**. It mentions various kinds of media – including those that are termed all-purpose, selective and differential. Experiment 4 makes use of these media as we determine **two kinds of counts**:
   a. "**Total aerobic plate count**" with <u>Plate Count Agar</u>, an **all-purpose medium**. (Actually, "total aerobic" is a misnomer: We will not be able to count the total number of bacteria, and aerobic refers to the incubation conditions – not specifically to those bacteria that are termed "aerobic.") Choose the one plate that has between 30 and 300 colonies. Look for a roughly 10-fold difference in the number of colonies between the adjacent plates. If, for example, you get over 200 colonies on one plate, and the next one down the line has just a few, you can probably question the technique you used in mixing the dilutions.
   b. "**Total gram-negative plate count**" with <u>MacConkey Agar</u>, a **selective-differential medium**. (The 30-300 rule also applies here.) Note diagram on following page.

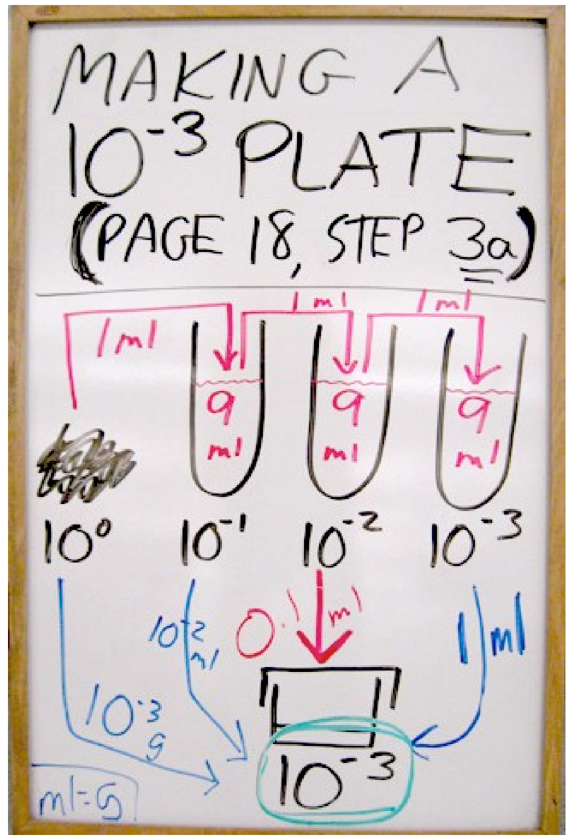
- **<u>Selective feature of MacConkey Agar:</u>** Bacteria that form colonies on this medium are **gram-negative**, as **the medium <u>inhibits gram-positive</u> bacteria**.
- **<u>Differential feature of MacConkey Agar:</u>** This medium **differentiates** between colonies of lactose-fermenting organisms and non-lactose-fermenting organisms:
  - First of all, it should be mentioned that bacteria generally produce an alkaline reaction when they grow on organic media such as MacConkey Agar and the others we use in lab. The reason: **Deamination** of **amino acids** by bacteria occurs which releases ammonia which is an **alkaline** product.
  - Colonies of cells that **ferment** the sugar (lactose) in the medium produce a lot of **acid**, and colonies of these organisms have a **<u>net</u> acidic reaction** (the acid over-neutralizes the alkaline reaction) which is detected by the pH indicator in the medium turning **red**.
  - Colonies of cells that do not ferment lactose will have the basic **alkaline** colonies (which are **whitish**).

3. Back in step 3a of Period 1, the question is asked "**Why are these plates marked 10^{-3}**?"

   a. A "10^{-3} plate" would mean that the plate was inoculated with one ml of a 10^{-3} dilution. However, the equivalent of that particular amount of inoculum can be achieved with other amounts of other dilutions. (Recall that you did something like this in Chemistry when adjusting molarities and amounts of solutions.) Thus, a "10^{-3} plate" can be achieved in various ways as shown in the diagram below. We made a 10^{-3} plate by inoculating 0.1 ml from a 10^{-2} dilution.

   b. In the Appendix C formulas, this value is called the "plated dilution." Also from this diagram, you see that the plated dilution (in this case, 10^{-3}) represents the actual amount of undiluted hamburger that is being tested – namely, 10^{-3} gram! With our equipment, we would never be able to spread such a small amount onto a plate, so we do the next best thing which is dilution and plating the equivalent amount.





EXPERIMENT 5C – PERIOD 1

1. **This is an exercise where the concepts of cells, CFUs, colonies, incubation and dilution theory all come together – along with another exercise in proper aseptic technique**. We will also touch on scientific notation and logarithms, keeping it as straightforward as possible.

2. We will be taking samples of a growing culture (one whose population of cells is increasing) at different time points. The plate count method will be used to determine the no. of CFUs/ml at each time point. Using averaged data from the entire class we should be able to create a growth curve. An explanation of the way we graph a growth curve is shown on the first page of Experiment 5C.

3. So, each pair will use one sample that had been taken from a growing *E. coli* culture at (a certain specific time point) and determine the **CFUs/ml** and also the **absorbance** of that culture.

   a. Finding **CFUs/ml** by the dilution plating method should be "old stuff" to us by now.

b. **Absorbance** is determined with the aid of a spectrophotometer which sends a beam of light into a culture. (Most likely the use of spectrophotometers was covered in a chemistry course.) The spectrophotometer passes a beam of light through the culture, and a decrease in the amount of light emanating from the culture reflects a population of cells that are absorbing the light. The less light detected by the spectrophotometer, the higher the absorbance reading, and therefore one assumes the higher the concentration of cells. We expect the absorbance reading and the CFUs/ml to rise along with the age of the culture during the exponential phase.

4. We can clarify the procedure on the board or handout. Note the table of recommended dilutions for performing the dilution plating (which need to go out further for cultures that have been growing longer). **One ml amounts** will be plated, so (unlike the hamburger experiment) the "plated dilution" will be the same as the dilution of the sample. **Always indicate the "plated dilution" on the plates.**

FOR NEXT TIME:

1. Be sure to have read the introduction to Experiment 7, and note in the procedure how everyone is going to ultimately observe all of the known cultures as well as his/her assigned "unknown."
2. Keep up with the schedule and announcements on the website which are posted periodically.