LAB LECTURE NOTES FOR WEEK 1

Note that these lecture notes are generally written from or for the **instructors' point of view**, but all information is applicable all around.

HANDOUTS FOR TODAY

1. The tentative **schedule** for the semester which indicates the second week as "Virtual Week."
2. **Excerpts from lab manual** covering material for today – Experiment 1, Period 1 and Exp. 3A, Period 1
3. Our **microscope handout** which covers both kinds of microscopes is now in the lab manual as pages 90A and 90B.
4. The official **syllabus** is the same as the Fall, 2013 syllabus which was accidently included in the present manual by the printer. Everything on that syllabus still applies for Spring, 2014.

INTRODUCTION TO MICROBIOLOGY 102

1. Introductions of personnel.
2. Brief statement about Microbiology 102 and its overall organization, purpose and goals, expanding the introductory material in the syllabus – along with a summary of the type of organism we will be studying. The following two points are included.
   a. As you will probably be going over in the Microbiology 101 course, bacteria constitute a large "domain" of living things, distinct from the other two biological groups. Some major differences are indicated on the following table which may be gone over more in lab.

"DOMAIN"	CELL TYPE [1]	PEPTIDOGLYCAN? [2]	MICROORGANISMS?
Bacteria	prokaryotic	present	**all** (bacteria – including the cyanobacteria)
Archaea	prokaryotic	absent	**all** (archae, formerly called archaebacteria)
Eucarya[3]	eukaryotic	absent	**some** – including yeasts, algae and protozoa.

[1] The prokaryotic cell type is one in which there are no membrane-bound organelles within the cell which is the case for the eukaryotic cell type. These organisms are referred to as **prokaryotes** and **eukaryotes**, respectively.

[2] **Peptidoglycan** is found in the cell walls of bacteria and will undoubtedly be discussed in the lecture course.

[3] The Eucarya include all of the plants and animals as well as the separate groups of protozoa, algae and fungi.

   b. As mentioned in the Syllabus, it is important to note that in our course we are only working with the **relatively small universe** of bacterial species that are "easy to grow" in the lab. They are the type of organism that (depending on the particular species) can (1) grow well in food and possibly cause spoilage, (2) grow well in us and possibly cause disease, (3) participate in various stages of biodegradation, and (4) "contaminate" things in general. Many of these are very easy to isolate from nature as we will find out in Experiment 11 – and even in our hamburger experiment which we start in the fourth week. What we learn from the organisms that we study in Micro 102 lab can apply to bacteria in general (even those you heard about in the news or elsewhere that can't be cultured in a lab at all) and also higher forms of life. Unlike other introductory microbiology courses, we go beyond the usual **chemotrophic** bacteria (those that respire and/or ferment to obtain energy) and also study **phototrophic** bacteria (those that get energy from light). We are filling in "the big picture" as you will see as the course progresses – not memorizing a bunch of trivia that we can forget about after each quiz.

3. Purchases:
   a. By next week, you will need to get the **Lab Manual**. The cost is **$10.00**, and it is available from the Microbiology Club. We keep the cost down by producing our manuals ourselves and having them printed locally. Otherwise you pay **much more** for a manual from one of the big publishing houses, and these tend to be superficial and full of exercises irrelevant to our course.
   b. You will also need to purchase some **microscope slides** and also a **glass marking pencil** like the kind we have available to a limited extent in the lab today. Rather than buy them in a large quantity which is how they generally come in bookstores, our Microbiology Club has a package of 20 slides and a marking pencil available for **$5.00**.

4. Items at your desk today:
   a. Schedule and First Week handouts (as mentioned above).
   b. Also a microscope slide (with already heat-fixed smears) that will be stained and observed.
   c. Petri dishes, swabs, tube of sterile saline.

5. Our on-line materials:
   a. Our **website** at http://www.jlindquist.com/microbiology102/ includes **course updates** which could be considered as a supplement to the lab lectures. There is also a special section for **links** to the virtual experiments and also to various resources (such as lab lecture notes and summaries of microbiological concepts).
   b. We will utilize **Learn@UW** for posting grades securely and privately.

6. Some helpful direction to keep in mind:

> As a general rule in **preparing for the next lab session**, always **check the schedule** well ahead of time, and read through the relevant material in the manual. In the **items due column** of the schedule, there may be a relevant Appendix to have read before lab. Also, you will find supplementary material about the various concepts listed in the **links section** of the website's homepage; the explanations given there (including videos) are sometimes more easily understood than what may be found in the corresponding part of the manual.
>
> We hope to review the essentials about the experiments in the lab after they are finished. Also, note that a link to the lab lecture notes for any particular lab session is posted on the website's homepage soon after the lab has met; often a "rough draft" is posted ahead of time.
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> As for the **virtual laboratory exercises** (also indicated on the schedule), clicking on the link to any of these labs where they are listed on the website's homepage will take you directly there. In the lab manual is additional information about each of the virtual labs as well as space for recording results. In the regular lab sessions, we find time to review various essential things regarding the virtual exercises.

FURTHER INTRODUCTORY COMMENTS

1. **Our attendance policy**:
   a. We expect you to show up for every lab session as your lab partner and your observations and understanding of the results and concepts of your experiments depend on it. If you miss two consecutive lab sessions without notice, we will assume you have dropped the course.
   b. Let us know if – for sickness or any other reason – you cannot attend lab. **Before e-mailing the instructor with questions about what was missed and what to do to make it up**, consult the schedule and the manual – and make sure that you see the results of the procedures done in your absence when you get back. We can save the things you inoculated and also afford the opportunity to start any "unknown" you missed getting on the day you were absent. And note that the Lab Lecture Notes will always be posted on-line (sooner or later, hopefully sooner).

2. Stress **safety and cleanliness**:
   a. Accidents and insurance: Students should not assume there is a blanket insurance policy for them at the UW. Hopefully they have their own insurance situation OK. Instructors can be sued if a serious hazard (which should be obvious to the instructor) is not pointed out before it happens!
   b. Fire drills and actual fires: Generally we go out the door to the right and keep turning right until we are out the door. We always need to get way out into the parking lot and not stand by the building.
   c. **NO EATING, DRINKING OR GOING SHOELESS IN THE LAB!!** We are always concerned about hand to mouth transfer of bacteria (which could be hanging around from a spill), and the floors are never guaranteed clean of broken glass.
   d. Just added in a recent semester is this admonition: **NO RUNNING IN THE LAB!**
   e. Mention about washing hands, disinfecting benches, etc.
   f. **Leave excess outer garments, bookbags, etc. in lockers** in the hall. Be sure to keep your valuables close by.

3. How to use the token-operated lockers in the hall.

4. Some things about the lab:
   a. Note contents of drawer: loop, needle, forceps, etc.
   b. On the bench – at least two sets of each of these at the table for four (called the "pod"): stains & reagents, burners, strikers, disinfectant squeeze bottle, etc.
   c. Which microscopes to use – something to make note of for the entire semester:

> **For Stained Smears** we use the **microscopes in the cabinets** between each pair of students. For these scopes (which we call the "regular light microscopes") we have the handout provided today – easy directions to keep on hand as needed. Instructor will demonstrate light control and focusing and also the use of the oil-immersion lens.
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> **For Wet Mounts** we use the **phase-contrast microscopes** which are set up by the instructor in the middle and back of the lab. Instructor will **demonstrate** how to use: Basically it's just insert slide and focus, making sure that you **start with the 40X objective lens as close as possible** to the slide and then focus up with the fine adjustment while looking through the microscope.

   d. More items around the lab: replacement stains/reagents (by the not-too handy sink), discard area, **incubator room**, fire/safety equipment (extinguishers, eyewash, fire blanket, etc.). Regarding the incubators, each section has assigned shelves in the incubators which hold the metal trays in which we place our tubes and plates for incubation. These incubators are generally held at 30°C and can be adjusted to refrigerate the tubes and plates after an appropriate incubation period.

5. **Today's agenda – the three things detailed on the handout for today**: (1) making and observing a wet mount of living organisms, (2) sampling the environment, and (3) staining and observing smears of bacteria. THESE THINGS CAN BE DONE IN ANY ORDER. (That would help to keep the lines short behind the phase-contrast scopes which are limited in number.)

EXPERIMENT 1 – PERIOD 1, STEPS 1-3

MAKING AND OBSERVING WET MOUNTS

1. Microscopic observation of **living organisms** is the object here. For this we prepare a "wet mount" and find it most advantageous to use the phase contrast microscope.

2. In the "hay infusion" (which is sometimes actually a straw infusion), the water leaches organic materials from the hay such that we get a dilute solution of organic compounds which bacteria can utilize as nutrients. Consequently, protozoa can utilize bacteria as their nutrients. Photosynthetic organisms such as green algae (coming from the lake water) may also be seen.

3. How to prepare wet mount. Here is where we use the cover slips. **Instructor demonstrates** the vaseline-sealing technique which helps to keep the wet mount from drying out (smearing a very small amount of vaseline on the palm of the hand and then lightly scraping it onto the four edges of the cover slip). Vaseline may not be required if the slide is going to be looked at right away.

4. How to use the "**phase-contrast microscope**." Using **only the 40X objective lens** which is **already in place**, just insert the slide, carefully focus – starting with the lens almost touching the coverslip, and observe.

One must take care not to allow the stage clips to scrape across the face of the objective lens. Raise the objective lenses (or lower the stage) to insert and remove the slides.

EXPERIMENT 1 – PERIOD 1, STEPS 4-6
SAMPLING THE ENVIRONMENT

1. **Brief mention of major concept**: Everything in this environment is contaminated with micro-organisms. We can see this for ourselves from the results of this experiment – including how "dirty" the air in the lab is. Most laboratory facilities are more up-to-date than this lab regarding airflow and dust control. From this experiment, we see how we will have to be careful during the semester such that we don't contaminate our bacterial cultures with organisms from the air and surrounding objects.

2. **Some important terms in the introduction that must be understood at the beginning**:
   a. The basic **cell** (meaning the "vegetative cell" as opposed to other types of cells such as endospores and reproductive spores) is **the unit of life for all bacterial species**. Cells of most species of bacteria reproduce by binary fission – that is, they "multiply by dividing" ($1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16$ and so on).
   b. After a cell lands on the medium in a petri dish, it will start to reproduce and result in a **colony** of cells – if it can utilize the nutrients in the medium. So, a **colony** is a discrete mass of millions of cells that is visible to the naked eye, originating from a common source such as one cell.
   c. However, a group of cells (2 or more which land on the plate together) can give rise to one colony. Therefore, **we say whatever originates the colony is a "colony-forming unit**." A spore is a colony-forming unit if it can germinate into a vegetative cell to start the process of dividing.

3. **Incubation of the plates**: The 30°C incubator provides an environment suitable for many different types of organisms. The medium in the petri dish allows for "growth" (increase in number) of many of the common contaminating organisms. The individual cells will divide by binary fission and form visible colonies which we will be able to see with the naked eye after incubation. As a rule, petri dishes are incubated "upside down" (i.e., medium side up).

EXPERIMENT 3A – PERIOD 1
STAINING AND OBSERVING BACTERIAL SMEARS

1. When preparing smears of bacterial cells on slides, where the cells are fixed in place, the cells tend to appear transparent when viewed with the microscope. So we need to **stain** them in order to discern their shapes.

2. Students are each provided with slide of two smears, each being a smear of a certain species of bacteria. The slides need to be **heat-fixed** so they stick to the slide during the staining, rinsing and observing processes. **Demonstrate the heat-fixing process** – using forceps to pass slide through flame 3 times. ***(Note: The smears may have already been heat-fixed prior to being passed out. If so, we will do our first heat-fixing the next time we meet in lab.)***

3. **Staining process** is detailed at the end of today's handout. Must be done **in sink**, not on the bench. Note that **no coverslips** will be used in the preparation or observation of the stained smears.

4. When one prepares a smear (as we will do next time), we allow the drop of cells to dry out. As the water is gone, all enzymatic activity ceases, including processes which can degrade the cell. **A smear can then last <u>indefinitely</u> such that it can be stained and observed long after initial preparation.** Throughout the semester we depend on that fact such that we can get a lot of stuff done in timely fashion and then have better time to stain and observe our slides.

5. Always use the "**<u>regular light microscope</u>**" (from the cabinets) to observe the stained smears. Procedure is given on handout, but some things need to be pointed out (i.e., **demonstrated**) now:

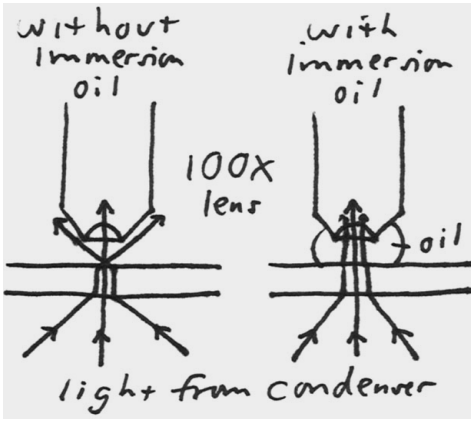
   a. How to carry microscope – **always with two hands**!

   b. Unravel just enough cord to plug it in. (Not to have cord dangling over the side of the bench.)

   c. Point out various parts of scope (which are also on the diagram on the microscope hand-out): **ocular and objective lenses** (total magnification is obtained by multiplying the two individual magnifications), **coarse and fine focusing knobs**, **condenser** (focuses light onto the specimen; always positioned <u>all the way up</u>), **iris diaphragm**, **stage and stage clips**. How to position the slide with the stage clips should be mentioned.

   d. **Demonstrate focusing technique** with coarse and fine focusing knobs as per handout. **Make sure you are focusing on the top surface of the slide. Bottom surface of slide should be cleaned off completely** before observing slide. It is too easy to do initial focusing (with low-power lens) on "dirt" on the bottom surface, and then when it comes time to use the high-power oil immersion lens, one can scrape that lens into the slide and possibly cause some damage. Initial focusing is done with 10X objective lens; then use the 100X (oil immersion) lens for the "official view."

   e. Explain use of **immersion oil** which is used only with the 100X objective lens (the oil immersion objective). As this lens is extremely small, we need to have enough light to get through/into it, so a drop of immersion oil between the lens and the specimen (no cover slip on specimen!) prevents scattering of light which would occur otherwise. Immersion oil has the same refractive index (ability to bend light) as the glass slide. And, as it is called **immersion oil**, the lens is **immersed** in the oil!! (Lots of times we may find that the lens is hovering high over the oil and the specimen from doing too much turning of the focusing knobs.)



   f. When finished with the microscope: (**1**) all oil must be wiped off the stage and lenses (using only lens paper on the lenses – no paper towel!!), (**2**) the green (10X) lens should be clicked in place, and (**3**) the stage should be centered such that nothing sticks out which can be jammed when putting the scope back into the cabinet.

6. Terms to be aware of:

   a. **MAGNIFICATION**.

   b. **RESOLUTION**: The ability to see separate objects **as separate objects**, not a blurry image which may suggest just one object.

   c. **PARFOCAL**: The ability to stay in focus when switching from one objective lens to another. When we switch from the 10X objective to the 100X (oil-immersion) objective, we are almost in focus. When we move the fine adjustment up or down (up to a quarter-turn of the knob), we should achieve a sharp view of our cells.

IN CONCLUSION

1. One can start with any of these three activities. This will help prevent backups behind the limited number of phase microscopes.
2. Reminder about **discarding slides**, and this is already in the printed directions:
   a. **Wet mounts**: **Not to pick them apart**! Just dump the whole thing into the **disinfectant**. Disinfectant tray is available on the center table.
   b. **Stained smears**: Discard in **red buckets** on center table.

NEXT WEEK IS "VIRTUAL WEEK!" (Week 2):

- No in-lab session.
- Details will be explained on a website update on Thursday or Friday, January 23 or 24.

FOR THE NEXT TIME WE MEET IN LAB (Week 3) – Read over the following:

- Introductions to Experiments 1, 2 and 3.
- Appendix B.
- Page 117.