Project 1: Introduction to Microbiology Laboratory

Microscopy, Photo-microscopy, White Blood Cells

Lab Main Page:

<http://www.scienceprofonline.com/vmc/vmc-lab/vmc-laboratory-photomicroscopy-wright-stain.html>

Readings:

Links to the required readings can be found on VMC Main Page for this lab. See class schedule for related textbook reading.

Purpose:

The purpose of this laboratory is to introduce students to the expectations and safety procedures in the microbiology laboratory. Students will learn the proper use of the microscope and how to take pictures using the microscope camera.

Outcomes:

### After you complete this lab, you will be able to:

* Apply the rules of laboratory safety to keep self and others risk free during laboratory procedures.
* List and describe all the rules of laboratory safety.
* Construct a model outline of the expectations of the laboratory.
* Evaluate the potential risk of a procedure and construct a plan to minimize the risk.
* Use the laboratory microscope, camera and laptop computer effectively to collect laboratory data.
  + Correctly focus the binocular, compound light microscope on all objectives.
  + Take pictures using the microscope camera and correctly label them for use in laboratory reports.
  + Save data to the appropriate computer drive to later access and share data with lab partner.
* Become familiar with the formed elements of blood: red blood cells, white blood cells, (lymphocytes, neutrophils and monocytes).

Introduction to Laboratories

This semester, you will carry out several laboratory projects. Some will be completed in one lab session, whereas others will require two or more lab sessions. During some sessions, you will be working on more than one project at a time. (See Course Schedule.)

Attendance

You are expected to attend all lab sessions. Check the Course Schedule against your calendar and make certain this class fits into your plans. There are no opportunities to make up for missed labs. Missing a lab session for lab projects that are carried out over multiple sessions will result in a loss of 50% of the points possible for that lab report. Attendance in lab **requires proper lab attire**: lab coat, safety glasses (provided in the lab), closed toe shoes, long pants, hair tied back.

Preparation

If you want to finish each laboratory in the allotted time, you must come *completely* prepared. Preparation is important for your safety and the safety of your fellow investigators. Preparation for the following session will consist of:

1. Laboratory Main Pages: These are found on our class website, the Virtual Microbiology Classroom. http://www.scienceprofonline.com/virtual-micro-main-2.html

2. Readings: These are listed at the beginning of each project. Read them *before* lab.

3. Pre-Laboratory quiz: These can be found on Moodle. These must be completed before you arrive for the lab in which you start a project.

4. Be certain you have lab coat, closed-toe shoes, safety glasses and hair tie-back (if necessary). If you are unable to participate in lab because you are not properly attired you will be ineligible for the points for the laboratory report (see attendance policy).

Lab Reports

Part of what you will learn through the laboratory portion of this course is how to write a scientific lab report. During the semester your will practice writing portions of lab reports, and well as complete lab report. All lab reports must be typed, double spaced and formatted using the APA style. The lab report consists of developing an introduction and hypothesis, materials and methods, reporting results, analyzing the data, and summarizing your study in the conclusion. The lab report must be in your own words, and have a reference section where you cite your sources in APA format. The goal in developing this approach to lab reports is to prepare you for using a scientific approach in your healthcare field. Not all sections of the lab report will be completed for each lab.

* **Introduction**: Summarize your reading and provide a purpose for performing the exercise.
* **Hypothesis:** This section will allow you to use the information in your reading to suggest an outcome for the laboratory procedure. We form hypotheses prior to testing and this gives us an expected outcome based on what we know. This information will allow us to evaluate our controls and tests.
* **Methods:** This sections includes a detailed description of how you did the lab; detailed enough so that someone else could replicate your procedure. Make sure this section (as well as your entire lab report), is in your own words, not just sections copied and pasted from the lab project instructions.
* **Data**: This section provides for an organized, systematic method of recording and reporting your observations. Providing this evidence is necessary for critical review of your work – by yourself and your co-workers.
* **Analysis:** This section will allow you to draw conclusions about your data. Again, a co-worker (or instructor!) can follow your analysis based on the evidence you have provided. You will refer to recorded data as you analyze your results. Errors in analysis may be detected through this critical review.
* **Conclusion:** Using your analysis of the data you will draw a conclusion from your procedure relative the hypothesis you developed. In this section you will include a discussion of why you think your results are valid and any potential sources of error.

YOUR LAB REPORTS MUST BE PRINTED OUT IN COLOR, AS MANY OF THE LAB TESTS YOU WILL LEARN ARE INTERPRETED BASED ON THEIR COLOR.

Laboratory Final Exam

At the end of the course, there will be a laboratory final exam to test your ability to use techniques and strategies learned throughout the semester. Objectives and assessment measures can be found on Moodle.

Scientific Method

The scientific method is essential to microbiology. It provides a reliable way to ask and answer scientific questions by making observations and doing experiments. It is important for your experiment to be a fair test. A fair test requires that only one factor (the variable) be changed during the experiment and all other conditions be kept the same.

Your laboratory reports will include each of the steps in the scientific method. Because many of the laboratory projects are designed to illustrate a principle or teach you a technique, sometimes you may have to observe carefully to see the steps in a particular laboratory, but they are all there.

A brief outline of the steps in the scientific method follows:

1. **Ask a Question**
2. **Do Background Research**
3. **Construct a Hypothesis**
4. **Test Your Hypothesis by Doing an Experiment**
5. **Analyze your Data and Draw Conclusions**
6. **Communicate your Results**

Rules of the Laboratory for Biology 130 (Microbiology)  
Lab DOs:

1. Read all material assigned for the week in the textbook, laboratory manual and online sites before you come to lab. If it appears you do not know what you are doing in lab you will be asked to leave.
2. Store all backpacks, books, purses, coats, etc., in the cubbies at the back of the room.
3. If you are pregnant or are immunosuppressed or are taking immunosuppressant drugs, discuss your decision to take this class this with your physician **immediately**. A list of potential bacteria used in the lab can be found in syllabus.
4. Record all data needed for lab projects using the laptop computer provided in the lab. Store your data on your “O” drive and/or email all saved data to yourself or your lab partner.
5. Tie back hair and secure it from touching your face.
6. Before and after each lab, disinfect your work space and wash your hands.
7. Clean and store microscopes properly after each use.
8. Report any improperly stored equipment on the lab equipment log.
9. Report any accident or injury to the instructor *immediately*.
10. Wear a lab coat, closed-toe shoes and goggles at all times during the experiment.
11. Turn off your Bacti-Cinerator® IV and Bunsen burner when they are not in use.
12. Know the location of and how to use the fire extinguisher, fire blanket and eye wash station before you begin your work.
13. If you spill a culture, cover the spill with a paper towel, soak the towel in disinfectant and allow it to sit for 20 minutes. Dispose of the toweling in a biohazard bag.
14. Adhere to the directions for disposal of all items used in the lab.
15. Observe universal precautions (see below) at all times.

Lab DON’Ts:

1. Do not touch your face, eyes, or hair during lab.
2. Do NOT consume food or beverage in the lab.
3. Do not place anything in your mouth.
4. Do not take anything out of the lab. NO personal belongs may come to the laboratory space; nothing from the laboratory may be removed. Any personal item brought into the lab will be autoclaved before it can be returned to you. (For instance, this means if your phone ‘accidentally’ comes into the lab it will be disinfected by being exposed to temperatures of 121o C at 15 psi of pressure for 20 minutes)
5. Do not wear scrubs to class. These are work clothes and should be reserved for your work environment.
6. If you are feeling ill, do not work with live microbes.

Universal Precautions: SAFETY FIRST!

Safety is essential in laboratory work. Due to the highly infectious and lethal nature of agents potentially found in body fluids, we must be extremely cautious. It is your responsibility to ensure that:

1. No one comes in contact with your body fluids. Properly dispose of any material that is contaminated with your body fluid immediately.
2. You do not come in contact with another person’s body fluids.
3. You always wear a gown, gloves and safety glasses when engaging in risky laboratory procedures such as obtaining or handling blood products.
4. You do not practice “ophthalmologic virology.” In other words, don’t look at the people next to you and make a judgment about whether or not they are safe based on appearance.
5. You assume all body fluids are contaminated with a lethal disease and act accordingly.

*Note:* Failure to follow laboratory rules puts you and others at risk of injury. Points will be deducted from your lab report for a breach of the laboratory protocols & safety precautions. If it appears you do not know what you are doing or you fail to follow directions in lab you will be asked to leave.

Penalty points will be assessed on your laboratory practical for any failure to strictly adhere to the rules of laboratory safety.

**Microscopy**

Principles of Microscopy

You will use the microscope in various exercises throughout the course. A specific microscope is assigned to the station where you sit throughout the term, and it is your responsibility to take good care of it and use it correctly. Each time you use your microscope, **you** will make an entry on your lab card, recording the number of the microscope you used. When you retrieve your microscope if you find it not in proper storage order, note that in the instructor’s log on the designated clipboard.

You will be working with a **compound light microscope**. Magnification is the result of two lenses: an objective lens and the ocular lens. The objective lenses, located on the rotary nosepiece, achieve four different degrees of magnification:

|  |  |  |
| --- | --- | --- |
| **Name** | **Characteristics** | **Magnifying power** |
| scanning power | shortest objective, red stripe | 4X |
| low power | next shortest, yellow stripe | 10X |
| high-dry power | intermediate length, blue stripe | 40X |
| oil immersion | longest, white and black stripe | 100X |

The ocular lens, located at the end of the body tube, has a magnification power of 10X. The **total magnification (TM)** is determined by multiplying the power of the objective by the power of the ocular. For example, 4X times 10X = 40X. Using two sources of magnification makes this microscope a **compound** microscope.

You should be very cautious when using the oil immersion objective and immersion oil. First, be sure you are using immersion oil, and not another kind of oil. Second, **only the oil objective (100X objective) should come in contact with the oil**. All the other objectives on your microscope are non-oil objectives and will not work properly if they get oil in them, which will happen if they are dragged through oil on a slide. **Be sure you do not move from an oil immersion lens to a 40X objective, because the 40X objective will drag through the oil and be ruined.** The oil needs to be cleaned from the oil-immersion objective after use, and this is done using special paper (lens paper) that will not scratch the lens.

Your microscope is referred to as an on **light** microscope because the specimen is observed using visible light. The light source is an incandescent bulb that is turned on by the toggle switch at the side of the base. For proper illumination, adjust the iris diaphragm, which is located just under the stage. The diaphragm lever controls the amount of light that enters the condenser and facilitates optimum observation of contrast and depth of field. In general, close the diaphragm on low power (this reduces the amount of light reaching the stage, preventing “burn out” of the image), and open the diaphragm for the higher power objectives.

The term **field of view** refers to how much of your sample, or the area of your sample, you can see at any one time. The field of view decreases with increased magnification. Notice this effect when you look at the items to be examined in this lab. Be sure to center your specimen, using the stage adjustment lever, before you increase magnification so that what you want to observe will still be in the field of view.

The objectives on a microscope are said to be **parfocal** if you can change from one objective to another and still have your specimen in focus without having to focus more than a little. This is a very convenient characteristic of a set of lenses, because as the magnification increases, the depth of field decreases. If you had to find your specimen using a high-magnification objective, it would probably take quite awhile, because you would tend to miss the specific position where the specimen is in focus. With parfocal lenses, you can find your specimen using low-power, long depth-of-field lenses, and then switch to a high-magnification objective, knowing your specimen will be in focus (or very close to it) under the high-magnification objective.

When focusing, you will see that high magnification objectives (40X and 100X) come very close to the slide. There is very little **working distance** with these objectives. When using them, be sure you use only the *fine* focus knob.

As two small objects appear to move closer to each other, a point is reached where the eye is unable to distinguish the objects as separate entities, and only a single object can be observed. The smallest distance at which two points can be seen separately is called the **resolving power** of the lens. The resolving power of the human eye at ten inches is 0.1 mm. This **resolution** (Figure 2-1) increases if you use the microscope to aid your eye, and increases more as magnification is increased. However, there is a limit to the resolution of even the highest magnification lens on a light microscope. Other types of microscopes, such as electron microscopes, must be used to resolve smaller structures of bacteria (e.g., flagella) and viruses.

Resolved Unresolved

**Figure 1.1**: Levels of Resolution

As the light from your light source passes up through the slide from below and then enters air again, the light is bent and goes off to one side or the other, rather than continuing on through the lens and to your eye. This bending (Bauman p. 96, Figure 4.2) is called **refraction** and occurs any time light passes from material of one density to material of another density. When refraction occurs, the refracted light is lost, and it is harder for you to see your object. Refraction can be partially overcome if material of the same density is placed between the slide and the glass lens of the objective. Modern light microscopes use a particular type of oil for this purpose, and a particular kind of objective (Bauman p. 99, Figure 4.5). These objectives are referred to as **oil-immersion** objectives and are meant to be used with immersion oil – without oil, you can see very little through them.

In many of the exercises during the following weeks, it will be important for you to see individual cells under the microscope. As you look at things under the scope today, distinguish those things that you are supposed to observe from any **artifacts** – irrelevant things seen because of the way the specimen was prepared. In this lab you will look at a preparation of the dye malachite green. This preparation contains dye molecules in solution, but also contains aggregates of un-dissolved dye. You will see these aggregates in the dye preparation in this project, and you also may see dye aggregates when you perform other stains. You should learn to distinguish these aggregates, which are artifacts, from your specimens. Be able to explain how you distinguish your specimen from artifact.

We will investigate the formed elements of blood by observing a Wright’s stain of human blood. We will focus on the identification of the white blood cells known as neutrophils and lymphocytes because they are key players in the immune response to infectious disease agents. Neutrophils are phagocytic cells capable of diapedesis. They have a large dark staining segmented nucleus that takes on many different shapes. For this reason, neutrophils are often referred to as ‘polymorphs’ or ‘segs.’ The generally make up 50-70% of the white blood cells. Lymphocytes are smaller than neutrophils and lack the cytoplasmic granules found in neutrophils. They commonly have large dark staining nuclei and a small rim of cytoplasm. Lymphocytes make up 20-40% of white blood cells.

PROCEDURES: Project 1

1. Retrieve your microscope.

a. Every student has a microscope assigned to the specific seating location in the laboratory. On the bench in front of you there is a number. Find the microscope with the corresponding number in the microscope cabinet and bring it to your bench.

b. Remove the dust cover and store it in the cabinet drawer. Make certain when you remove the dust cover that the nosepiece is positioned such that the red scanning power objective is locked in place and the stage is in the lowest position. Using lens paper, check to make certain the 100were x oil-immersion lens was stored with no oil on it. If your scope is not stored under these conditions, find the microscope log and record your findings.

c. Plug the scope into the power strip under the bench and use the toggle switch to turn on the light.

2. Locate a Wright’s stain of human blood from the slide box on your bench.

a. Slide the stain into the stage clip so the slide is secure.

b. Check that you have performed this correctly by using the ‘X’ and ‘Y’ controller to move the slide around the stage.

3. Click the scanning power (4X) objective into place on the nosepiece (it should already be there).

a. Use the mechanical stage control to position the specimen over the light source.

b. Use the coarse adjustment to raise the stage to its highest position. Then, as you look through the microscope ocular, slowly turn the coarse adjustment away from you, lowering the stage until your specimen comes into focus. Remember to adjust the iris diaphragm for the best image. A labeled picture of the microscope is posted on Moodle for reference.

c. Fine tune your image with the fine adjustment knob.

d. Click the low power (10X) objective into place. You will probably have to use only the fine adjustment knob to focus your object well, because the objectives are parfocal.

e. Click the high-dry power (40X) objective into place on the nosepiece. Using ONLY the fine adjustment knob, fine-tune the image.

f. IMPORTANT: When focusing, always start at low power and work your way up. This not only helps you find and focus on specimens quickly, but also diminishes the potential of ramming a long, high-powered objective through a slide when trying to focus with the coarse adjustment knob under high power. Pay attention to the **working distance** of the lens. This is the distance between the lens and the slide when the specimen is seen in sharp focus; the higher the magnification, the smaller the working distance. To avoid ramming a long objective into a slide, observe the **Working Distance Rule**: ***Use the coarse adjustment knob* *on low power only*.**

4. Find a neutrophil on the blood smear. Take a picture of this white blood cell:

a. Attach the camera to the laptop computer using the USB cord.

b. Focus the microscope on your specimen.

c. Open the LAS EZ software from the desktop. When the program opens there will be three tabs at the top of the page: Acquire, Browse, and Process. The Acquire tab will be highlighted when the window opens.

d. Click the “Acquire Image” at the bottom of the left of the Acquire column.

After you click ‘Acquire Image’ you will need to indicate the objective under which you are observing the image.

e. Select the ‘Process’ tab.

f. Select the ‘enhance’ tab and crop:

* + 1. Under the crop tab click start, select the area you wish to crop, click ‘finish’ and ‘apply’. If you wish to save this image, after clicking the ‘apply’ icon, you will have the option of saving the image or replacing the image.
    2. Select ‘replace’

g. Using the ‘replaced’ image, select the Annotate tab from the Process tab.

* + 1. Select ‘line’ and click ‘show’
    2. Select ‘distance line from the drop down menu on the right side of the ‘line’ box.
    3. Use the cursor to draw a line the width of the cell you wish to measure.
    4. From the ‘Actions’ tab, select ‘Merge’; when the Leica Application Suite pop-up window asks, “Are you sure you wish to merge the annotation with the image?” select ‘yes’.
    5. You may wish to use the line function to add arrows to the photograph tagging important features (i.e. nucleus, cytoplasm, Gram positive coccus, etc). Repeat the process selecting ‘arrow’ from the Line tab. In the ‘label’ space of the Line tab box you can add your title and then position the arrow with the text box in the appropriate place on the photo.
    6. Save the image by selecting the save icon from the right side of the screen. Then, select the copy tool, copy the picture.

5. Save the picture in a Word document so that it may be inserted into your lab report.

a. Open a Word document and create a text box.

b. Paste the image from the LAS EZ it in the text box.

c. Label the text box with the following:

* + 1. Label (i.e. Figure 1)
    2. Title (e.g. Neutrophil with Wright’s stain)
    3. Total Magnification
    4. Date

1. Repeat the procedure to take a picture of a lymphocyte.
2. Use the X/Y controller to navigate in the pattern indicated in **Figure 1.2** to avoid counting the same area twice. Identify each white blood cell you encounter until you have counted 50 WBCs. Record the cell counts in a table similar to Table 1.3
   1. Figure 1.2: Pattern for Examination of Slide

Feather Edge



* 1. Table 1.3: Cell counts of patient \_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Neutrophils | Lymphocytes | Monocytes | Eosinophils | Basophils |
|  |  |  |  |  |

7. Store your data on your student ‘O’ drive and email a copy of your data to your lab partner.

8. Storing the microscope:

When putting your microscope away, you need to be sure it is in proper storage order. Proper storage order consists of the following:

a. The microscope stage is clean.

b. The oil is cleaned from the oil immersion lens and all other parts of the microscope.

c. The 4X objective is clicked into place.

d. The finger cot is covering the 40X objective.

e. The stage is in the *lowest* possible position.

f. The dust cover is on.

g. Return your microscope to the proper storage space in the microscope cabinet.