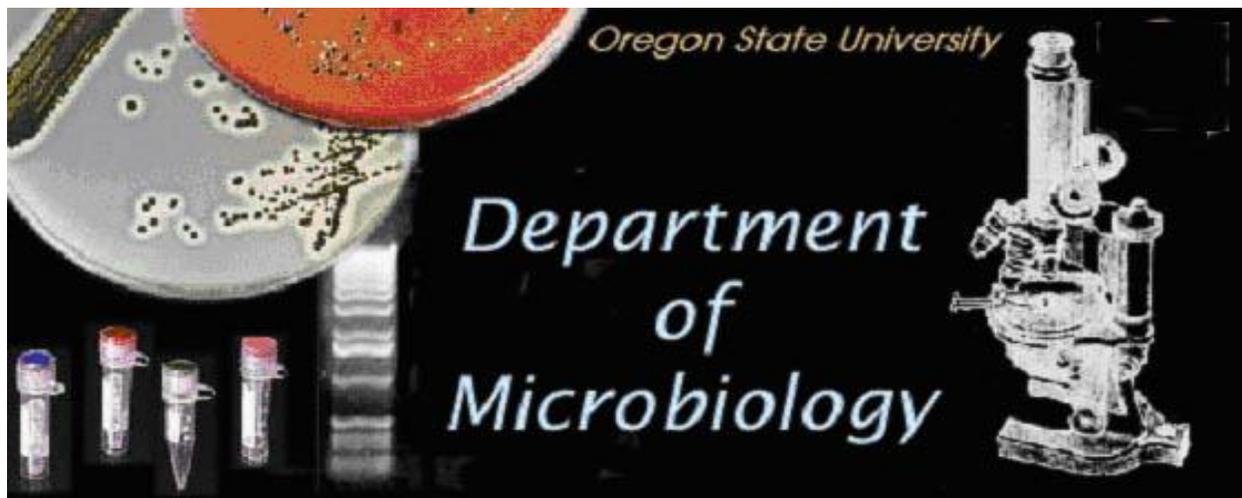


General Microbiology Lab Manual

MB 303

Fall 2018



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Instructors: Dr. Allison Evans - Monday & Thursday labs
Dr. Linda Bruslind - Tuesday lab

Note: All MB 303 labs will start Week 1 (First FULL week of classes)

Monday section - starts on Mon., Sept. 24, 1 pm.

Tuesday section - starts on Tues., Sept. 25, 1 pm.

Thursday section - starts on Thurs., Sept. 27, 1 pm (no lab Sept. 20).

****All MB 303 labs have a no-show drop policy. If you don't attend your first lab in week 1, you will be dropped from MB 303.**

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MB 303 GENERIC EXAMPLE SCHEDULE

****Please note that the schedule for your specific term will be provided to you in class and may not follow the schedule below****

	Date	Activity	Notes
Week 1	Tues., Apr. 3 or Thurs., Apr. 5		Introduction to the Lab
		Exercise 1	Use of the Microscope
		Exercise 2	Simple Stains: Microbial Morphology
		Exercise 3	Transfer of Bacteria
		Exercise 4	Environmental Sampling (take-home)
		Safety Quiz	Mandatory 100% (online, due before next lab)
Week 2	Tues., Apr. 10 or Thurs., Apr. 12	Exercises 1, 2	Lab Notebook Due
		Exercise 3	finish
		Exercise 4	continue
		Exercise 5	Differential Stains
		Exercise 6	Pure Culture: Streak Plates
		Exercise 7	introduction
		Exercise 8	Scientific Literature (homework assignment)
Week 3	Tues., Apr. 17 or Thurs., Apr. 19	Exercise 7	Dilution Scheme Due
		Exercise 8	Assignment Due
		Exercises 4 & 6	finish
		Exercise 7	Quantification of Bacteria
		Exercise 9	Culturing the Unculturable
Week 4	Tues., Apr. 24 or Thurs., Apr. 26	Exercises 3, 4, 5	Lab Notebook Due
		Midterm	Ex. 1-6, 8 (35 minutes)
		Exercise 7	finish
		Exercise 10	Culturing Bacteria: Nutrition & Environment
		Exercise 11	Introduction to the Spectrophotometer
Week 5	Tues., May 1 or Thurs., May 3	Exercises 6, 7, 9	Lab Notebook Due
		Exercise 10	Finish (class discussion of results: ≈1.5 h)
		Exercise 13	Identification of Unknowns (start; lab #1)
		Exercise 12	In-lab worksheet due before leaving lab
Week 6	Tues., May 8 or Thurs., May 10	Exercise 13	Gram – and Gram + Flow Charts Due
		Exercise 12	Growth Curve (data sheet due before leaving lab)
		Exercise 13	Continue (lab #2)
		Exercise 14	Respiration/Fermentation/Anaerobic Respiration
Week 7	Tues., May 15 or Thurs., May 17	Exercise 12	count plates (data sheet due before leaving lab)
		Exercise 13	Continue (lab #3)
		Exercise 14	finish
		Exercise 15	Additional Tests for Gram Positive Bacteria
		Exercise 16	Additional Tests for Gram Negative Bacteria
Week 8	Tues., May 22 or Thurs., May 24	Exercises 10, 11	Lab Notebook Due
		Exercises 13, 15, 16	finish (Ex 13 is lab #4)
Week 9	Tues. May 29/ Thurs. May 31	Exercise 12	Assignment Due
		Exercise 13	Unknown Reports due
			Hand in as 3 separate items with name/seat # on each
Week 10	Tues., June 5 or Thurs., June 7	Exercises 13, 14, 15, 16	Lab Notebook Due
		Lab Final	Ex. 1-16 (1.5 h)

All items are due at the start of student's scheduled lab day, unless otherwise indicated. Items not handed in by the start of lab on scheduled lab day will be marked as late (see Course Policies on pg. vi for details about late policy).

LEARNING OUTCOMES & LEARNER EXPECTATIONS

Learning Outcomes*:

After successful completion of MB 303, students will be able to:

1. Properly prepare and view microbiological specimens for examination using brightfield microscopy.
2. Use pure culture and selective techniques to enrich for and isolate microorganisms, using proper aseptic technique.
3. Estimate the number of microorganisms in a sample using both viable plate counts and spectrophotometric methods.
4. Evaluate a microbiological problem in the context of an unknown microorganism, using appropriate media-based methods for identification.
5. Accurately document and report observations and interpretations made during laboratory exercises.
6. Use appropriate microbiological lab equipment and methods, in order to conduct and analyze experimental measurements relevant to microbiology.
7. Practice safe microbiology, using appropriate protective and emergency procedures.

Learner Expectations:

1. Attend lab (on time) and stay until all lab exercises are completed.
2. Read laboratory exercises in lab manual before they are to be performed.
3. Bring lab manual and lab notebook to class.
4. Come already prepared to take exams (i.e. do not wait until the night before to cram).
5. Participate in learning activities and complete tasks on time.

*All MB 303 learning outcomes have been derived from the American Society for Microbiology (ASM) *Curriculum Guidelines for Undergraduate Microbiology*, Part 2: Competencies and Skills, Microbiology Laboratory Skills, published Sept. 2014.

http://www.asm.org/images/Education/FINAL_Curriculum_Guidelines_w_title_page.pdf

BASIC COURSE INFORMATION

Instructors

Dr. Allison Evans, Nash 326, Allison.Evans@oregonstate.edu

Dr. Linda Bruslind, Nash 322, bruslindl@oregonstate.edu

Office Hours - During lab period or by appointment (email instructor for available days/times)

Pre-requisites/Co-requisites – MB 302

Laboratory Supplies (required by each student)

MB 303 Laboratory Manual (available for sale at OSU bookstore)

Bound lab notebook (i.e. spiral notebook or composition book)

Long-sleeved lab coat, either fabric or Tyvek (available for sale at OSU bookstore)

Grading (approximate, subject to changes as necessary)

EXAMS.....75 pts.

LAB NOTEBOOK.....150 pts.

UNKNOWN.....100 pts.

IN-CLASS POINTS & ASSIGNMENTS.....75 pts.

TOTAL.....400 pts.

Points can be deducted at any time for not following instructions and/or safety rules as indicated in the MB 303 lab manual.

Final grades are assigned on a straight percentage basis. These percentages reflect traditional numerical rounding upwards, and no additional rounding will be made at the end of the term: A = 92.5-100%; A- = 89.5-92.4%; B+ = 86.5-89.4%; B = 82.5-86.4%; B- = 79.5-82.4%; C+ = 76.5-79.4%; C = 72.5-76.4%; C- = 69.5-72.4%; D+ = 66.5-69.4%; D = 62.5-66.4%; D- = 59.5-62.4%; below 59.5% = F. For S/U grading a 70% (C-) or above is required to receive an "S". Election of S/U grading should be known only to the student and their academic advisor.

Format

Each lab will begin with a lead-in lecture about the principles for the exercises and demonstrations of new techniques. **Students are required to read the exercises before coming to lab each week**, to optimize understanding, performance and allow for completion of the exercises during the allotted time. Ask questions when you do not understand a laboratory procedure. Good laboratory technique depends not only on knowing **what** you should be doing but **why** you should be doing it.

Some exercises require independent work, and others will be done in pairs/groups. However, each student must perform his/her own observations, drawings, and write-ups for each exercise, which is to be recorded in a bound lab notebook.

Lab coats

Each student must provide their own long-sleeved lab coat, to be left in the lab for the duration of the term or brought by the student each week. **Students without a lab coat will be asked to leave the lab.**

Care of Valuables

Items of value should **not** be brought to the lab because of danger of theft or damage. The Department of Microbiology is not responsible for personal items brought to lab.

Accommodations of Disabilities

Accommodations for students with disabilities are determined and approved by Disability Access Services (DAS). If you, as a student, believe you are eligible for accommodations but have not obtained approval please contact DAS immediately at 541-737-4098 or at <http://ds.oregonstate.edu>. DAS notifies students and faculty members of approved academic accommodations and coordinates implementation of those accommodations. While not required, students and faculty members are encouraged to discuss details of the implementation of individual accommodations.

COURSE POLICIES

- **Lab safety/lab procedures:** Students are expected to follow the rules for laboratory safety & procedures described in detail on pages ix-x. An online safety quiz (on Canvas) must be taken before the start of the 2nd lab. Each student may take the quiz as many times as necessary. A perfect score (100%) is mandatory or the student will not be allowed to continue in the lab.
- **Attendance policy:** Attendance in lab is mandatory. This is a 2-credit lab-only class that lasts only 10 weeks. You *must* attend to warrant receiving credits for this class because you cannot learn the hands-on part of the lab if you are not physically in the lab. During the 10-week term, you may miss one (1) lab only and still receive a passing grade. If you have more than 1 absence, you will receive an “F” in this course.
- **What constitutes an absence?** Students are expected to be on time and fully participate for the scheduled lab time.
 - Arriving at any lab session *more than 30 minutes late* to lab will be considered a full (i.e., one, 1) absence, even if you are present for the rest of the lab session.
 - Arriving at lab *more than 10 minutes late* (but not more than 30 minutes late) counts as half (0.5) of an absence. Arriving at lab more 10 minutes late (but not more than 30 minutes late) a *second* time counts as *second* half (0.5) of an absence. If you arrive between 10-30 minutes late on two separate occasions, you have accumulated one (1) full absence. A third tardy arrival results in a failing grade for the course. If you have already missed one lab and arrive more than 10 minutes late, you have accumulated 1.5 absences, which results in a failing grade for the course.
- **What happens if I have to miss a lab?** Labs **may not** be made up outside of scheduled MB 303 lab times. In-class points **cannot** be earned if you miss a lab. Contact the instructor as soon as possible if you missed or will miss a lab to talk about consequences in detail.
 - If you know of a reason that you will have to miss a lab in advance, **attending a different MB 303 lab section may** be possible but is **not** guaranteed. Attending another MB 303 lab section is possible *only with prior permission of your instructor and the instructor of the other lab section*. Email a request to *your* instructor as soon as you are aware of a time conflict with your scheduled lab time. If you know you have to miss a lab in advance *and* you secure the permission of the instructor to attend another lab session *in advance*, this is not counted as an absence and you are eligible to earn your in-class points.
- **What happens if I have a serious, unforeseen emergency that is out of my control?** Exceptions to the course policies will be made only in the case of truly serious extenuating circumstances (i.e. serious illness, hospitalization, death in the family, car accident) that are *documented*. The instructors retain the right to decide if circumstances are extenuating or not. If you would like to ask for an exception for extenuating circumstances, you *must* be able to provide documentation of the circumstances, such as a doctors’ note, a death certificate/obituary, or a police report. If you have an extenuating circumstance but do *not* have proper documentation, you are *not* eligible to receive an exception to the course policies. If you would like to be considered for an exception, contact the instructor as soon as is practical. Do not wait a week to ask for an exception. If you are granted an exception, you and the instructor will work out the details of the exception. An exception is NOT the same as an excused absence. There are *no excused absences in MB 303*, regardless of the reason. Any student can miss one lab, regardless of the reason, and still receive a passing grade. *No student* can miss more than one lab, regardless of the reason, and pass the class with a *letter grade*. Most frequently, extenuating circumstances are handled by awarding an *incomplete*, which you must make up in the following term. If you are granted an exception in which the solution is receiving an incomplete, your grade will be an I/[grade], where [grade] reflects your grade in the class, excluding the missed work. You are *not* eligible for an incomplete if your grade in the class, with the exception of the missed work, is an “F”. In that case, regardless of the extenuating circumstances, one receives an “F” in the class. In all cases of documented extenuating circumstances, *your best option is to contact the instructor as soon as possible*.
- **Missed exams:** No make-up exams will be given. Missing an exam will constitute a zero.
- **Correspondence:** emails sent to instructors or TAs must be sent using a student’s ONID account.
- **Lab Notebooks:** page **Error! Bookmark not defined.** describes expectations for the lab notebook.
- **Submission Policy:** Students are allowed to submit an assignment **once** for grading. Components of partially completed assignments are not eligible for the late policy once the

assignment has already been handed in and graded. This applies to the lab notebook and external assignments.

- **Late Work:** all items are due at **the start of a student's scheduled lab (1 pm Tuesday or Thursday)**, unless otherwise indicated on the syllabus. Items handed in before the end of a student's scheduled lab on the date due will have 10% deducted [ex: you earn 90 / 100; 10% of 100 is 10: your grade = $(90-10)/100=80/100$]. Items handed in by T 1 pm or R 1 pm on the day following the due date will have 25% deducted. Items more than 24 hours late will **not** be accepted.
- **Grading:** students have **2 weeks** from the time that papers/exams are returned (or available for pick-up) to contest a score. Please look your papers over carefully! Points will be updated on Canvas every 1-2 weeks by the lab TAs. Check to make sure all your grades are recorded correctly. **If a grade is listed incorrectly or not posted, a student should contact their lab TA as soon as possible.**

Prohibited Academic Misconduct:

<http://studentlife.oregonstate.edu/studentconduct/academicmisconduct>

Students are expected to be honest and ethical in their academic work. Academic misconduct is defined as any action that misrepresents a student or group's work, knowledge, or achievement, provides a potential or actual inequitable advantage, or compromises the integrity of the education process. It includes:

- cheating- use of unauthorized materials, information, tools, or study aids
- falsification- fabrication or invention of any information.
- assisting- any action that helps another engage in academic misconduct.
- tampering- interfering with an instructor's evaluation of work by altering materials/documents.
- plagiarism- representing the words/ideas of another person or presenting someone else's words, data, expressed ideas, or artistry as one's own.

When evidence of academic dishonesty comes to the instructor's attention, the instructor will document the incident, permit the accused student to provide an explanation, advise the student of possible penalties, and take action. The instructor may impose any academic penalty up to and including an "F" grade after consulting with his/her department chair and informing the student of the action taken.

All persons must treat each other with dignity and respect in order for scholarship to thrive. Behaviors that are disruptive to teaching and learning will not be tolerated and will be referred to the Student Conduct Program for disciplinary action. Behaviors that create a hostile, offensive or intimidating environment based on gender, race, ethnicity, color, religion, age, disability, marital status or sexual orientation will be referred to the Affirmative Action Office.

LAB NOTEBOOKS

The lab notebook is an important part of your grade. Notebooks will be collected and graded throughout the term, as noted in the Laboratory Schedule.

Each student must have a lab notebook designated for MB 303 alone, such as a spiral bound notebook. A lab notebook with carbon copy pages is not required. The notebook should be clearly labeled with the student's name, lab day, and seat number on the front cover. Pages in the lab notebook should be numbered at the top. The first page of the lab notebook should be used for a Table of Contents. Each exercise should be listed, followed by the page numbers that correspond to each exercise. The table of contents must be kept up to date.

Points will be deducted if your lab notebook is not neat, organized and legibly written. In a professional laboratory, a researcher's lab notebook belongs to the lab and must be written so that other people can read and follow the work. Write clearly and legibly; if your writing is illegible, you will not receive credit. Your notebook should also be well organized. If you run out of room at the end of an exercise, reference the rest of the exercise to a later page in your notebook by writing in large print at the bottom of the page, i.e. The Rest of Exercise # is on Page #. You should have plenty of room in your notebook - do not crowd things. If results will be collected later, leave several blank pages for the results. Finally, there should be no loose pages in your notebook. All inserts, such as computer graphs, should be stapled into your notebook (do not use paper clips). It is to your benefit to make sure the grader can find with ease all the pertinent information.

Each lab exercise includes two parts, Objectives and Results, which should be written directly into your lab notebook. Items that need to be done outside of lab (i.e. graphs) should be well labeled and stabled into the lab notebook.

Objectives should be written into your lab notebook **before** you start the lab. Objectives are a series of statements describing the purposes of the exercise and the concepts you will be learning in lab. You should know what your objectives are before you start each lab. There are examples of Objectives for Exercises 1-4 in the lab manual. **Each student is responsible for writing Objectives for Exercises 5-7, 9-11, 14-16 in his/her lab notebook.** Objectives do not need to be written in the lab notebook for Ex. 8, 12, or 13.

The **Results Section** includes all lab observations, interpretations, calculations, and graphs. **All observations made during lab should be written directly into your lab notebook during lab time.** Observations include not only a written description but often an interpretation or drawing of the observation as well. All drawings and descriptions should be well labeled. Therefore, a specimen viewed under the microscope should always include the total magnification, a drawing depicted as microscope field of view (i.e. a representative of what you see when looking in the microscope), and a detailed description of the relevant specimen(s) viewed. Parts of the specimen(s) should be labeled if there are parts that can be labeled, and all other pertinent information should be included. Scientific names must be written in correct binomial nomenclature (genus capitalized, species lowercase, both words underlined). Calculations and graphs can be done on your own time but must be included in the results section. **You may re-write any observations that you want for your own use (i.e. re-do a table on computer outside of lab), but your grade will be based on your initial handwritten observations & interpretations made in lab and entered into your lab notebook. In order to receive full credit, be sure to use the lab time to make all the necessary observations and record all of the results that are indicated, in your lab notebook.**

As a final note: You should always look over your lab notebook after grading to make sure the grader did not make any mistakes and that point totals were added correctly. The policy of this lab is to give you a maximum of two weeks to get any grade changed starting the day the graded item is handed back (or available for pick up). This applies to all items in the class.

LABORATORY SAFETY RULES & PROCEDURES

- 1) **CLEAN** desktop with **DISINFECTANT** (i.e. quaternary ammonium compound, QAC) at the **beginning** and **end** of class. Carefully wash hands with soap before leaving the lab.
- 2) **KEEP YOUR WORK SPACE CLEAR** – keep only the lab manual, your lab notebook, and any necessary lab supplies on your bench top; everything else should be placed under the bench, keeping the aisles clear. Backpacks/coats/cell phones etc should not be left on the lab bench top. **Do not bring valuables to lab!**
- 3) **DO NOT** eat, drink, chew gum or tobacco in lab. Open beverage/food containers must be left on the hallway shelf outside lab. Keep your hands out of your mouth, nose, and eyes.
- 4) **WEAR APPROPRIATE CLOTHING** – Each student is required to provide and wear a long-sleeved lab coat at all times in lab. The lab coat may be left in the lab for the duration of the term or brought each week. Students without a lab coat will be asked to leave lab. **“Loaner” lab coats are not available.** Closed-toed shoes are required. Protective eye goggles & gloves will be provided if deemed necessary. Lab coats are required at all times when a student is in MB 303 lab, but should be removed whenever a student leaves the lab room (i.e. to visit the restroom, etc).
- 5) **PAY ATTENTION TO ANNOUNCEMENTS** - No MP3 players/iPods in lab; turn off cell phones/pagers while in lab.
- 6) **NO UNAUTHORIZED VISITORS** in the lab. **NO ANIMALS** in the lab.
- 7) **KNOW THE LOCATION** of the fire extinguisher (on the wall in the hallway), the fire blanket (the red box on the wall near the windows), & the eye wash (yellow capped faucet at sink). A full body shower is located outside the lab, to the right and around the corner, at the entrance to the media room in Nash 324. A partial body shower is located at the front desk in Nash 304.
- 8) **INJURIES** – report accidental cuts or burns to the instructor or TA immediately. If the injury needs professional assistance, you will be escorted to the Health Center or proper facility.
- 9) **BROKEN GLASSWARE** - Call the instructor or TA to assist you. Do not dispose of any glassware in the regular garbage cans.
 - a. **Contaminated glass** is placed in the large metal can on the discard table.
 - b. **Non-contaminated glass** is placed in the large cardboard “broken glass” box.
- 10) **SPILLS** – If you spill anything in lab, inform the instructor or TA so that they can assist you in proper clean-up. If culture is spilled on your clothing or belongings, they may require decontamination to assure your safety.
- 11) **INCUBATING OF CULTURES** – each workspace has an assigned number that can be used to identify your materials. Carefully label all materials to be incubated with your name/initials, seat #, and organism identification. Place materials to be incubated in the incubation tub at the front of the lab, unless otherwise directed.
 - a. Label **culture plates** on **agar side** with your name/initials, seat #, and organism identification. Place plates in incubation tubs **agar side up**. This prevents moisture from forming on the inside of the lid and obliterating colonies.
 - b. Label **culture tubes** on the **glass (not plastic caps)** with your name/initials, seat #, and organism identification. Do **not** use Sharpie™ to label glass items, since it does not wash off in our dishwasher. Place tubes in racks in incubation tub.
- 12) **BUNSEN BURNERS** in the lab have almost invisible flames – use caution when they are on. Long hair must be tied back during lab to avoid contact with flame. Make sure to completely turn off your Bunsen burner when finished. Alert the instructor or TA to the smell of gas in the lab.

- 13) **PIPETTING** – Do not pipette by mouth. When using rubber pipette bulbs, insert the pipette gently into the bulb, holding the top of the pipette, to avoid breaking the pipette and potentially cutting yourself. Dispose of pipettes in the pipette collection containers.
- 14) **DISCARDING CLASSROOM MATERIAL** – All materials used in lab that are contaminated with culture (tubes, plates, pipettes, etc) must be autoclaved before cleaning or disposal.
- a. **Used microscope slides** should be disposed of in the cardboard box for glass waste.
 - b. **Used cover slips** should be disposed of in the cardboard box for glass waste.
 - c. **Used razor blades/pins** go in the metal can for contaminated glass waste.
 - d. **Plastic Petri plates** go into an autoclave bag at the discard table. Do not discard **glass** items in the autoclave bag. **Remove all rubber bands before discarding plates.**
 - e. **Plastic transfer pipettes, swabs, etc** go into an autoclave bag at the discard table. Do not discard **glass** items in the autoclave bag!
 - f. **Glass pipettes** should be placed in the plastic containers located on the lab bench.
 - g. **Culture tubes** go into wire baskets in metal containers (“coffins”) at the discard table.
 - h. **Glass bottles** go into metal containers (“coffins”) at the discard table. Loosen screw caps before autoclaving. Side-arm flasks should be filled with water first.
 - i. **Glass Petri plates** go into metal containers (“coffins”) at the discard table.
 - j. **Uncontaminated** paper towels used to clean desk with disinfectant or lens paper can be placed in metal container on the lab bench. The metal container should be emptied in the main garbage can at the end of each lab.
 - k. **Black wire racks** are to be returned to the lab shelves. Square colored plastic racks stay on the lab bench.
- 15) **MICROSCOPE USE** – our microscopes are valuable and must be cared for properly. When replacing the microscope after use:
- a. Clean all oil off the 100X objective, first with flat lens paper and then flat paper saturated with lens cleaner. Wipe dry with another piece of lens paper. Residual oil will destroy the seals on the objective. Crumpled lens paper will scratch the lens. Never use anything other than lens paper to clean your microscope lenses.
 - b. If you have gotten oil onto the 40X objective lens, inform the instructor or TA so it can be appropriately cleaned.
 - c. Clean off dirt or oil from the stage, condensers, or oculars.
 - d. Leave a low power (4X or 10X) objective into place.
 - e. Wrap the cord neatly around the oculars.
 - f. Roll out the microscope shelf completely before replacing the microscope. Sign and date the sheet in the cabinet. Have your TA check your microscope.
- 16) **LEAVING THE LAB** – Clear the lab bench of all cultures, plates, and other supplies. Empty metal can of paper waste into a large garbage can. Clean desktop with disinfectant and wash hands with soap. Make sure that you have all of your personal belongings. Please note that laboratory items (supplies, cultures, plates, VWR pens, etc) must remain in the laboratory.

Note: violations of laboratory rules and procedures may result in a point penalty at the discretion of the bench TA or lab instructor.