Micro 509 Laboratory Schedule for Spring, 2008

based on Nester *et al.*, 2006, 5/e, text & Leboffe & Pierce, 2006, 2/e, lab manual

This schedule was last updated on *Monday, March 31, 2008*

To view lectures (PowerPoint presentations) see Carmen

See www.phage.org/microbiology_schedule.htm for the lecture schedule
Your syllabus can be found at www.phage.org/school_syllabus.htm

For first day of lectures we will be covering chapters 1, 2, and 3

(through "Morphology of Prokaryotic Cells" on p. 53).

The following is an approximate lecture schedule for the quarter:

week	day	Tuesday	day	Thursday
1	1	chs. 0, 1, 2, & 3	2	chs. 3 & 4
2	3	chs. 4 & 5	4	ch. 5
3	5	midterm #1	6	chs. 6 & 7
4	7	chs. 7 & 8	8	chs. 8 & 9
5	9	chs. 9 & 10	10	midterm #2[1]
6	11	chs. 11 & 12	12	chs. 12 & 13
7	13	chs. 13 & 14	14	chs. 14 & 15
8	15	midterm #3	16	chs. 16 & 17
9	17	chs. 17 & 19	18	chs. 19 & 20
10	19	chs. 20 & 21	20	ch. 21
11	21	midterm #4	--	

The above schedule is to give you heads up on reading your text for the first and subsequent days of lecture.
Don't be ignorant: take the time to read your text as assigned, and read it well!

Micro 509 Grading Scheme

Midterm #1:	200 points
Midterm #2:	200 points
Midterm #3:	200 points
Midterm #4:	200 points
Lab exam #1:	50 points
Lab exam #2:	70 points
Lab participation and attitude:	20 points
Lab quizzes:	20 points
Lab cleanup:	20 points
Lab unknown:	20 points
Total:	**1000 points**

For lab schedule and directions, please proceed to the following…

[1] Starting with midterm #2, if we run into time trouble prior to a midterm then we will bump that midterm forward one day. We would be free to do this only once.

1. Read the first day's assigned reading. On page 6 of this document you will find instructions on material that you will be held responsible for on the first lab day (see those items starting with "1.1.2" which means lab 1, week 1, day 2). Please read the assigned material. This includes pages 1-5 of this document, pages 1-8 of your lab text, a section titled "intro to light microscopy" which can be found on p. 64 of your lab text (unit 3, lab 1, p. 64 is what "3-1.64" means), and then a bunch of material also found within this document. Note for the latter that you are asked to read a footnote and then Appendices 1, 2, and 3 found toward the back of this document. Please note that there are additional footnotes found throughout this document. These footnotes, each and every one of them, represent required reading for this course. Read them as you come to them. Yes, I know that reading footnotes is a pain, but it's the only way that I've come up with that will allow me to keep this document from becoming hopelessly cluttered.
2. This document isn't perfect. I'll try to inform you in a timely manner of any problems found within this document. Please let me know of any that you notice—often you will notice these things long before I do.
3. This isn't high school. Uncertainty and ambiguity are two painful aspects of biology, science, and medicine. In microbiology things will not always work. Get used to it. We will be working day in and day out with living things that don't always cooperate, and we will be learning challenging techniques that won't always provide immediate gratification. Furthermore, though I've seen plenty of people try to prove otherwise, it is next to impossible to do well in microbiology lecture or lab without making an effort to read your texts and supporting documents (such as this document). **If you don't want to be challenged—or are not equipped in terms of time or ability to do the necessary reading and other preparations for lectures and for labs—then don't take microbiology.**
4. Difficulty is no excuse for apathy. Your success in this lab and this course can be affected by your attitude. If you are fighting microbiology, convinced that taking microbiology is an unreasonable expectation imposed upon you, then you will not excel in this course. A key component to learning microbiology is becoming aware of how to do microbiology in the laboratory. If you are not paying attention—if you don't care—you only end up creating trouble both for yourself and for those who are counting on you.
5. Refer to this document (i.e., read the appropriate sections as indicated below) to have a clue as to what you need to be doing and how you need to be doing it in lab. You will be lost if you do not. In other words, follow all directions, instructions, and tips from your lab text unless those are contradicted by instructions found in this document. By all means seek help from your lab instructor if you are confused, but make sure that you have, at the very least, first looked for the information in your lab text **and** in this document.
6. This document contains footnotes. If you can't or won't read footnotes then there is no hope. All is lost.
7. Your lab grade is in part based on your lab participation and attitude. Remember, you need to be coming to lab, doing your share of the work, using proper (and appropriate) laboratory techniques, making meaningful observations of experimental results, and taking sufficiently detailed notes so that you have some sense, come exam time, of what you have done. Please also try to conduct yourself in a professional, courteous, civil, and approachable manner.
8. Your lab grade will also be based, in part, on learning your binomials. Starting the second lab (and then as indicated) you will be quizzed at the start of labs. Quizzes will consist, at least in part, of the equivalent of matching genus to species. You will be held responsible only for those organisms indicated as actually being used for a given lab. **These quizzes are not open book!** The purpose of this exercise is to familiarize you with the organisms you will be working with, and binomial nomenclature in general. BTW, a binomial looks something like this: *Escherichia coli* (note the italics and/or underlining as well as the fact that the first name, the genus, is capitalized, while the second name, the "specific epithet", is not—on exams, failing to properly underline binomials will be counted against your grade).

9. Your lab grade will also be based, in part, on understanding what you will be doing for a given lab. In conjunction with the your binomial quizzes you also may be quizzed on your appreciation of just what it is you will be doing in lab on a given day. **These quizzes <u>are</u> open book.** Yes, we do have an expectation that you will read your labs (and this document) prior to coming to lab, but note that the primary concern of these quizzes will be what you will be doing in the lab the day the quiz is given. That is, you should not beat yourself up over understanding the *interpretation* of procedures (e.g., what it means when colonies are red) unless you are instructed to start and complete a procedure that day (interpretation, however, will be an important component of lab exams as well as quizzes given on the days you will actually be interpreting—these distinctions are important because in microbiology you typically will be inoculating cultures on one day and then looking at them on another). Our hope is to motivate you to learn the material necessary to safely and efficiently perform the laboratory that you will be doing that day.
10. Your lab grade is also based, in part, on how thoroughly you clean up and otherwise keeps carts, counters, and drawers neat. See below for a discussion of proper cleanup procedures. This aspect of your grade will come from both your overall section's cleanliness and observations that are made of individual lapses on your part. Please contact your instructor if you observe any problems that you are unsure how to deal with.
11. Your lab grade is also based, in part, on your identification of your bacterial unknown. To ID your unknown you will need to attend lab on a regular basis, record data, and have some clue as to what you are doing in lab, but otherwise you should avoid spending sleepless nights worrying about this procedure.
12. Your lab grade will also include two open-lab-text exams. To do well in the lab portion of this course you should conscientiously complete the assigned laboratory exercises; record results, answer all questions (unless directed not to); and review your lab text (well) prior to taking the lab exam. In the exam you will be tested on lab results as well as your ability to perform specific techniques, but *in particular you will be tested on what results mean*. Lab exams are open-book and include questions assigned in advance (see "Your lab text has blue pages in the back," below). This style of lab exam keeps the focus on learning rather than on memorizing. Just imagine the alternative: a lab practical for which you have to memorize all of the material!!!
13. Read your lab text prior to coming to lab. You will understand what is going on better if you familiarize yourself with what you will be doing. Remember that your goal is to more easily do and understand the labs once you get to lab. Try to get a sense of what needs to be done, what materials you will need, etc. You may be quizzed, at the beginning of labs, on how well you have prepared.
14. Many lab exercises are performed over two or more lab periods. Taking note of what portions of your lab-text reading are appropriate to which day can be helpful to your understanding of exercises. For example, on day one of an exercise you typically will be asked to inoculate media whereas on day two you typically will be asked to interpret your results. Your level of understanding of the inoculation procedure obviously will be more relevant on day one whereas your understanding of interpretation obviously will be more important on day two. Any beginning-of-the-period lab quizzes will be geared towards these kinds of splits with inoculation emphasized on inoculation days and interpretation emphasized on interpretation days.
15. Labs will not begin early. Laboratory periods are set to begin at 2:00 or 4:15. Please show up on time to labs to take the lab quizzes. However, please refrain from entering or attempting to enter labs until the official start time for your laboratory section. Whatever you do, do not ask your lab instructor or anybody else found in association with the microbiology prep lab to let you into the teaching laboratory early. *Getting caught asking will result in 5 points being deducted from your grade (on a 1000-point scale)!!!* If you want to finish labs on time you should work diligently while in lab, choose partners who will also work diligently, and, above all, have a very good sense of what you will be doing in lab prior to starting. At the appropriate start of laboratory periods take the initiative to get yourself started by collecting reagents, setting up, and, to the extent that you can do so without instructor demonstration, performing procedures.
16. Place only a minimal amount of personal materials on your lab bench. Lab benches are places where contamination of belongings could occur. Please keep backpacks, extra clothing, extra books, and other

unused materials away from your lab bench. *Getting caught placing these materials on your lab bench will be deducted from your grade (1 point per incident on 1000-point scale).*

17. Working with test tubes. Move test tubes around in test racks, not by hand. Label tubes one at a time as you obtain them, so that you won't mix up reagents or cultures. Conscientiously flame tube openings as you remove and replace caps.
18. Work safely I. In addition to the safety instructions listed in your lab text, remember: (i) Be prepared and work with care. (ii) Do *not* knowingly harm yourself. (iii) Do *not* knowingly ingest any substance in the laboratory or cause others to do so, even if this is something that you have brought into the laboratory. (iv) Do *not* knowingly harm others. Violations of these rule (i) will result in deduction of points from your lab grade. *Violation of rule (ii) may very well result in your being reported to the university administration. Violation of rule (iii) will result in deduction of 5 points per incident from your grade (on 1000-point scale). If you violate rule (iv) you will be reported to the administration, and perhaps also the police!*
19. Work safely II. (iv) Avoid touching your face with your hands. (v) Wear disposable gloves when handling stains (i.e., as used for observing bacteria under a microscope; note that this is not an instruction to wear gloves at all times for all procedures though you certainly are welcome to do so should you feel compelled). (vi) Please keep burners burning only (approximately) as they are actually being used.
20. Do *not* return unused cultures and media to where you got them from unless you receive permission from your laboratory instructor to do so. Doing so could spoil procedures for other students. *Getting caught doing so will result in 5 points being deducted from your grade (on a 1000-point scale).*
21. Do your labs in the orders indicated in Table 1 (below). If you would prefer to deviate from suggested orders then please first discuss this with your instructor. There may very well be a reason for the order suggested, but not always.
22. Your lab text has blue pages in the back. These are questions associated with individual lab exercises. There will be an indication of which questions need to be answered in **Appendix 15**. You are responsible for these questions unless otherwise instructed. However, you do not need to hand in these questions. Instead, these questions will be found as part of your laboratory exam. Think of them as take-home possible exam questions (since that is exactly what they are). **Please be mindful that not only may these questions be a part of your lab exam, but also that your lab exam may also consist of questions that are *not* found on the blue pages.** Keep in mind that many lab exercises are done over two or more lab periods and that in many cases it may be more appropriate to answer these questions after you have observed and recorded your results.
23. If you have an idea about how a lab may be run better then please inform your instructor. We are doing our best to design labs so that diligent students can finish them in the allotted time. Please come to lab prepared to work quickly, efficiently, and safely, but let us know how we can improve procedures.
24. Make up labs may be extremely difficult or impossible to accomplish due to our use throughout these labs of living microorganisms. Therefore, please try hard to attend all labs.
25. Label all media and cultures, using marker on glass. These reagents will not be labeled for you and you will not be able to distinguish cultures without labels.
26. Use black, felt-tipped pens for most labeling. Save red Gram-stain permanent pens only when you will be destaining slides using organic solvents, e.g., when Gram staining. Note that the red ink is meant to be difficult to wash off and this makes it next to impossible to remove writing from tubes marked with Gram-stain pens.
27. Take care of your microscope. Only the 100x objective lens can be brought into contact with oil. Do *not* get oil on the 40x, 10x, and 4x objective lenses. Use alcohol and cotton swabs for most microscope cleaning. Use only lens paper or cotton gauze—never use Kimwipes® or paper towels—to clean the optics on your microscope. Do not even think of using paper towels to clean your microscope optics!!!!

28. These are various ways that media can be prepared. A *deep* is a regular tube filled with 10 ml (or in some cases, as specified, 25 ml) of agar media (use melted, not solidified; if used solidified this is *stab*). A pour tube is a large tube filled with 25 ml agar media (used melted). A shake tube also is a large tube filled with 25 ml agar media (also used melted).
29. Incubators. We typically will be using either a 25ºC or a 37ºC incubator. Some procedures will not work given a 37ºC incubation, so please keep track of what incubator you should be placing your cultures into. In many cases you will be asked to place your cultures on one or more trays which in turn will be placed in incubators for you. When incubations need to be completed before the next lab period, your cultures (if properly placed in the appropriate location) will be removed to a refrigerator. Where your text says 35ºC incubation we will be employing the incubator labeled "37ºC".
30. Make sure you stow your cultures in incubators properly and then clean up properly: See Appendix 1 for tips on how to how to clean up the lab and otherwise successfully complete your lab procedures.
31. Remember that putting forth "as little effort as possible" can make otherwise smart people look stupid. Often an absence of "getting it" can be attributed to mundane causes such as not reading (or recalling) this document, not reading (or recalling) your laboratory text, or not reading (or recalling) your lecture text. It's not that we don't want to help you, but part of helping you is helping you to learn how to help yourself.

TABLE 1: Summary of Labs[2]

#.wk.day[3]	day	unit-lab-page[4]	action	approximate lab name[5]	temp	length	#/grp[6]
weekend 0 / week 1 below							
1. **intro to micro**, 2. **molecules of life**, and 3. **microscopy**							
1.1.1	Tues	---	read	read pages 1-5 of this document	--	--	1
1.1.1	Tues	pp. 1-8	read	read introduction[7]	--	--	1[8]
1.1.1	Tues	3-1.64	start[9]	intro to light microscopy[10]	--	--	1
1.1.1	Tues	clean up	complete	see footnote[11]	--	--	1
3. **microscopy** and 4. **prokaryotic growth**							
2.1.2	Thurs	p. 13	read	aseptic transfers	--	--	1
2.1.2	Thurs	1-2.14	start	aseptic transfers[12]	25, 37	next	1
2.1.2	Thurs	2-15.61	start	effectiveness hand scrubbing[13]	25	48	2

[2] **The footnotes to this table are very important. Do not ignore them.**

[3] "**#.wk.day**" means lab number ("#"), what week of the quarter the lab is being run ("wk"), and what day (first or second; "day") the lab is being run.

[4] "**unit-lab.page**" refers to the location of the material in your book, e.g., "3-1.74" means unit 3, lab 1, starting on page 74. Please note that when a given lab is indicated to you then that means that you are expected to read that lab well, and to do so prior to coming to lab. You are also expected to determine what organisms (specifically, bacteria) are to be used in a given lab and then come to lab prepared to be quizzed on your ability to match genera with species e.g., *Escherichia* goes with *coli*. **Binomial quizzes will not be open book!**

[5] Labs (or readings) are presented in suggested order of initiation, though not necessarily in order of expected completion.

[6] "**#/grp**" is the group size that you will be working in for a given procedure. "1" means work alone; "2" means work in pairs, etc. Groups may vary depending on the number of students in a lab section.

[7] This lab will commence with a ~30-min intro to micro 509 lab by your instructor. You will then be given ~10 min to go over this document. You will then be given ~15 min to read the introduction found in your lab text on pages 1 through 9. Your instructor will then read pages 3-5 to make sure that everyone has seen/heard it (~10 min).

[8] All of today's procedures are to be done by every student (group size = 1). That means that it is OK to consult with your neighbors, but that the actual manipulations should be done by you!

[9] "**Read**" means that this is assigned reading for which you are responsible on the lab exam(s). "**Start**" means that the exercise will be begun but will or may need to be completed on a different day, which is particularly the case when cultures are inoculated during the first period and need to be observed during the second or third periods. "**Complete**" means that the entire exercise should be completed on the indicated day. "**Continue**" indicates that the exercise can or, more often, needs to be undertaken on the indicated day but is neither started nor finished on that day. "**Finish**" refers to the day that exercise will be completed.

[10] After reading the pages in your lab text associated with section 3-1, proceed with today's exercise by seeing **Appendix 2** and **Appendix 3** (below) for directions on microscopy in general and today's procedure in particular. (That is, see **Appendix 3** for description of what you will actually be doing in lab today.)

[11] Five to 10 minutes before end of this period refer to **Appendix 1** on how to clean up and otherwise complete a lab. Note that in subsequent labs you will not be explicitly prompted to clean up by this document, but of course that doesn't mean that you should not be cleaning your area and the lab in general at the end of all lab periods.

[12] See **Appendix 5**. Note that *not* resuspending cultures (e.g., as by vortexing) is a very common mistake.

[13] Make sure that you **do not** start out today's lab period by washing your hands. In addition, "it is best that you do not disinfect your workstation prior to this lab" since you will be using both your hands and your bench to explore

2.1.2	Thurs	3-1.64	complete	intro to light microscopy	--	--	1
weekend 1 / week 2 below							
4. **prokaryotic growth** and 5. **control of microbial growth**							
3.2.1	Tues	1-2.14	finish[14]	aseptic transfers	--	--	1
3.2.1	Tues	1-3.20	start	streak plate[15]	37	24-48	1
3.2.1	Tues	77-80	read	bacterial struct. & simple stains	--	--	1
3.2.1	Tues	3-4.81	complete[16, 17]	simple stains[18]	--	--	1
3.2.1	Tues	2-15.61	finish	effectiveness hand scrubbing	--	--	2
3.2.1	Tues	---	complete	phase contrast, *Pseudomonas*[19]	--	--	1
5. **control of microbial growth**							
4.2.2	Thurs	pp. 349-356	read	pipetting	--	--	1
4.2.2	Thurs	1-4.23	start	spread plate	37	24-48	1
4.2.2	Thurs	p. 217	read	quantitative techniques	--	--	1
4.2.2	Thurs	6-1.218	start	standard plate count[20]	37	24-48	2

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the effectiveness of hand scrubbing. Better yet, rub your fingers on the floor! Let your partner do the hand scrubbing if you suspect that you will have negative reaction to any of the various scrubs. Also, contrary to the instructions in your text, divide up your plate into five "quadrants" (0, 1, 2, 3, 4) and press your thumb post rubbing but pre washing/disinfecting to the quadrant labeled "0".

[14] "**Finish**" means that it is time to look at the cultures that you have been incubating, in this case, since the previous lab period, then to complete the questions found on the blue pages at the back of the book. Note that now is the time for you to try to fully understand what the procedure is all about and what your results are telling you. In particular, often there is material written in your lab text that won't make much sense until you see your cultures. Similarly, often your cultures won't make sense to you unless you reread the material written in your lab text. Don't forget to answer the questions associated with procedures found in the blue pages in the back of your book.

[15] See **Appendix 4** for tips on quadrant streaking. Note that the text's request to streak a mixture of *E. aerogenes* an *E. coli* is in error (at least according to the instructor's text). Use a mixture of *S. aureus* with *C. violaceum* and *S. epidermidis* with *S. marcescens* instead of the indicated combinations. Mix cultures by decanting. Place all mixtures in the "25°C" incubator.

[16] Don't forget to answer the appropriate questions found on the blue pages in the back of your book. You may be told not to answer certain questions. See **Appendix 15** to be sure. Note that answering questions in some cases will not be possible until you have completed a procedure.

[17] Note that for many procedures there is an indication of a "Lab One" and a "Lab Two" (and in some cases even a "Lab Three"). On the day you start a procedure it is important to understand what you will be doing for "Lab One." However, on subsequent days that you continue doing that same procedure it is equally important to understand what you will be doing for "Lab Two." It cannot be stressed too strongly that it will be abundantly obvious that you are not preparing for labs if you are stumbling around wondering what to do because you are not aware that there is a procedure indicated in your lab text for what to do on the day you complete a procedure.

[18] See **Appendix 4**. Don't do "cell dimensions" on p. 429. Indeed, as a general rule don't determine cell dimensions.

[19] When you have a few moments, e.g., while smears are drying, please observe the bacteria (*Pseudomonas*) under the phase-contrast scope at the front of the room (demonstration). Record what you see on p. 101 of your lab text. Make sure that your name is properly recorded by your laboratory instructor so that you will get proper credit for participation in this portion of today's lab.

[20] For your dilutions, at the tope of the p. 244 replace "10 µl" with "100 µl" and "100 µl" with "1000 µl". Use a 100 µl digital pipettor to transfer the 100 µl and a 1.0 ml glass pipet to tranfer the 1000 µl (1 ml = 1000 µl). Ask

4.2.2	Thurs	1-3.20	finish	streak plate	--	--	1
weekend 2 / week 3 below							
midterm exam #1							
5.3.1	Tues	9-1.282	complete	extraction of DNA[21]	--	--	2
5.3.1	Tues	2-14.58	start	use-dilution[22]	37	48	2
5.3.1	Tues	1-4.23	finish	spread plate	--	--	1
6. **metabolism** and 7. **DNA to protein**							
6.3.2	Thurs	3-6.86	complete	Gram stain[23][24]	--	--	1
6.3.2	Thurs	3-7.91	complete	acid-fast stains[25]	--	--	1

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your instructor for instruction as to how to use these devices. Note that you need to make sure that you have mixed dilutions well before removing volumes from them. Ideally this will involve employing a mechanical vortexing device. We will be employing the spread-plate technique for plating. Ask your instructor how to do this, including how to flame the "hockey stick" (which should be done by dipping the stick in alcohol and then immediately igniting the alcohol on the glass rod, without keeping the glass rod in the flame). See also pp. 28-30 for directions on how to perform a spread plate. **Keep flames away from alcohol. If your alcohol reservoir begins to flame, then please place the lid on it!** See **Appendix 8** and **Appendix 9** for tips on diluting and pipetting, respectively. Dispose of pipet tips in the proper, small plastic autoclave bags. Dispose of glass pipets in the supplied pipet bucket.

[21] For the DNA extraction procedure we will not be doing the optional follow-up spectrophotometry (which would employ the ultraviolet spectrophotometer and quartz cuvettes). We will be transferring 50 and 100 µl volumes using micropipetters and 1000 µl and 2 ml volumes using serological pipettes. Vortex and then tap down the proteinase solution before adding to bacteria. Remove three 50 µl volumes to your culture when directed. Note that there is a discrepancy between step 7 on p. 288 and Figure 8.1 on p. 289. Please cool down the bacteria *before* adding the Sodium Acetate. Add the cold ethanol, removing it using 5 ml serological pipettes.

[22] Choose a single bacterium-disinfectant combination to do this procedure. Your instructor will put a copy of Table 2.4 on the board so that you can choose a combination not also used by other members of the lab. If you are having trouble removing sterile beads then use a sterile loop to do so. Decant broth into pipet buckets to dispose of. Remember, if your bead is not completely submerged in disinfectant then it is not being disinfected over its entire surface. Ditto, if the bead is not completely suspended in water then it is not being completely washed. Pour supplied 25 ml sterile water blanks into sterile petri dishes to do this washing. Make sure that you employ free time during this procedure to proceed to set up subsequent procedures. Dispose of disinfectant down drains when done. Dispose of petri dishes into the red autoclave bag. See **Appendix 10**.

[23] We won't be Gram staining with *Moraxella catarrhalis*, just *S. epidermidis*, *E. coli*, and *B. subtilis*.

[24] See **Appendix 5** to review general procedures for smearing and staining. Your instructor will introduce you to Gram staining, emphasizing a description of how to properly destain: drop by drop until color stops visibly running out, then immediately rinse using tap water. Mark your slides using the red Gram-stain pens (not with black sharpies). Do not attempt to Gram stain smears until you are confident in your ability to make a smear in which individual cells are visible (i.e., not densely surrounded by other cells and not so diffuse that you cannot find any cells). Note that your goal in this exercise should be to reach a point where you are reasonably proficient in Gram staining your organisms; this means that you should do the procedure repeatedly until you are reasonably good at it (though be sure to move on to the next procedures while your smear is drying). *To give you incentive to both do this procedure well and to actually read these footnotes, please note that may will be held responsible for doing Gram stains to ID the Gram staining characteristics of a given culture on both lab exams.* Don't do "cell dimensions" on p. 433.

[25] Make sure you start this procedure while your Gram stain smears are drying and have the smears completed before actually doing your Gram staining. We will be doing the Ziehl-Neelson method (with hot plates under hoods) and *not* the Kinyoum method. The trick to getting the acid-fast stain to work is successfully heat fixing lots of acid-fast organism. Note that you are better off putting the carbol fuchsin on the slide *after* bringing the slide to

6.3.2	Thurs	---	complete	phase contrast, hay infusion[26]	--	--	1
6.3.2	Thurs	2-14.58	finish	use-dilution	--	--	2
weekend 3 / week 4 below							
7. **DNA to protein** and 8. **bacterial genetics**							
7.4.1	Tues	p. 107	read	selective media	--	--	1
7.4.1	Tues	4-1.108	start	mannitol salt agar[27,28]	37	24-48	4
7.4.1	Tues	4-2.111	start	phenylethyl agar	37	24-48	4
7.4.1	Tues	6-3.224	complete	direct count[29]	--	--	2
7.4.1	Tues	6-1.218	finish	standard plate count	--	--	2
8. **bacterial genetics** and 9. **biotechnology**							
8.4.2	Thurs	2-5.40	start	evaluation of media[30]	37	24-48	4
8.4.2	Thurs	4-4.116	start	endo agar	37	24-48	4
8.4.2	Thurs	4-5.118	start	eosin methylene blue agar[31]	37	24-48	4
8.4.2	Thurs	4-7.122	start	MacConkey agar[32]	37	24-48	4
8.4.2	Thurs	2-6.42	start	agar deep stabs	37	24-48	4
8.4.2	Thurs	2-7.44	start	agar shakes[33]	37	24-48	4

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the hot plate rather than before (since this stuff can spill off the slide as you walk with it). The Corning PC-351 hotplate should be adjusted to “high”. Note that the decolorizing agent is *acid-alcohol*, not the same decolorizing agent as that used for the Gram stain. You should decolorize with the acid-alchol extensively. Don’t do “cell dimensions” on p. 435.

[26] This is a demonstration found at the front of the room. You are required to view this demonstration.

[27] In groups of four you will then do a series of platings on various selective media. For plating of multiple cultures on a single plate, first divide up that plate into quadrants using a marker on the bottom of the plate; see p. 23 of your lab text for both instructions on how—and why—to do this as well as an illustration of this basic technique. Note that part of doing these exercises will be to practice your aseptic culture transfer techniques. That means that you should be performing all transfers that you are involved in doing all by yourself, though to reduce the total amount of transfers that you do you will divvy up transfers to be made among your group of four. Note that for these exercises we will also be employing a non-selective medium (nutrient agar) plates as positive controls. You don’t need to have more than one positive-control streak for any given stock culture, remember to divide up plates into quadrants, and please try to inoculate any *Proteus* species alone on a given plate (since *Proteus* tends to spread across the surface of plates).

[28] Note that a “Spot inoculation” involves dragging your loop on the surface of the agar for a centimeter or so. Refer to color images in your text for examples of what these should look like (e.g., Figure 4.9 on p. 128). You should read about how to do a spot inoculation on p. 23 of your lab text.

[29] Substitute *E. coli* for *Proteus vulgaris*.Your instructor will individually (or in pairs) introduce you to using the Petroff-Hausser and doing a microscopic total count. Otherwise, this procedure is done using the phase-contrast microscope found at the front of the room and we will not be employing the indicated stains unless cell motility turns out to be a problem. Your instructor will place the organisms on the counting chamber for you but make sure that you ask your instructor what dilution was employed before placing the bacteria on the slide. Please be kind to our phase-contrast scope. It will cost ~$6000 if we should need to replace it. Also be kind to the slides. They cost about $200 each.

[30] Use a loop to inoculate.

[31] Include an additional (4^{th}) inoculation of *E. coli*. That is, divide the plate four ways, with four streaks; there is no need to employ an additional EMB plate.

[32] It is in your best interest to inoculate the *Proteus* on a separate plate.

8.4.2	Thurs	2-10.49	start	effect of temp. on growth[34]	25,37	24-48	4
8.4.2	Thurs	4-1.108	finish[35]	mannitol salt agar	--	--	2
8.4.2	Thurs	4-2.111	finish	phenylethyl agar	--	--	2
weekend 4 / week 5 below							
9. **biotechnology** and 10. **prokaryote IDing**							
9.5.1	Tues	2-5.40	finish	evaluation of media	--	--	2
9.5.1	Tues	4-4.116	finish	endo agar	--	--	2
9.5.1	Tues	4-5.118	finish	eosin methylene blue agar	--	--	2
9.5.1	Tues	4-7.122	finish	MacConkey agar	--	--	2
9.5.1	Tues	2-6.42	finish	agar deep stabs	--	--	2
9.5.1	Tues	2-7.44	finish	agar shakes	--	--	2
9.5.1	Tues	2-10.49	finish	effect of temp. on growth[36]	--	--	2
midterm exam #2							
10.5.2	Thurs	3-5.84	complete	negative stain[37]	--	--	1
10.5.2	Thurs	3-9-97	complete	endospore stain[38]	--	--	1
10.5.2	Thurs	3-8.95	complete	capsule stain[39,40]	--	--	1
weekend 5 / week 6 below							
11. **prokaryote diversity** and 12. **eukaryote microbes**							
11.6.1	Tues	lab exam # 1	none	exam on first 10 labs[41]	--	--	1

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[33] The agar shakes will be found in a water bath (where they are being kept at approximately 45°C). Do not take agar shakes to your bench until you are just ready to inoculate them; otherwise they will have a strong tendency to solidify before you are ready for them. Rather than following directions explicitly.

[34] Note that we have only a single high-temperature incubator which will be set in the range of 50°C to 65°C (we will let you know which when we know). Don't forget that these cultures need to be placed on the proper-temperature trays.

[35] Don't forget that it takes reading your lab text to understand what you will be looking at. Take careful notes while making your observations and then to take time at the end of lab or after lab to complete exercises and back-of-book questions.

[36] Do the version that does not use a spectrophotometer. Recall that there are cultures in the incubators other than the 25°C and "37°C" incubators (e.g., 4°C and 50-60°C. Please ask for these cultures if you can't find them.

[37] We will look at these three organisms: *Micrococcus luteus*, *Bacillus cereus*, and *Aquaspirillum serpens*. Make a thick smear of *Aquaspirillum*. Place the smear at one end of the slide, not in the middle. Use a small drop of stain; if you use too much you will have trouble finding your organism. Make sure you mix the organism well with the stain. Try the following procedure if you can't get the one in the book to work: place slide cover on top of smear, put drop of India ink on one edge of slide, capillary action will draw the ink in and across the smear. Don't do "cell dimensions" on p. 431.

[38] We won't be using *Bacillus coagulans*. Don't do "cell dimensions" on p. 439. Otherwise fill in all blanks.

[39] Your instructor will introduce you to Pasteur pipettes at the beginning of the period.

[40] We will look at these three organisms: *Enterobacter aerogenes*, *Alcaligenes viscolactis*, and *Leuconostoc mesenteroides*. The capsule stain traditionally is quite difficult to pull off successfully so don't be too disappointed if you fail to observe a capsule (though, by the same token, don't give up too easily—it might take quite a bit of looking to see the organism, and make sure that you use oil to do that looking and that you have properly adjusted your iris diaphragm to maximize contrast without too-minimizing light). Serum is used to adhere cells. Use 20µl per smear. We will employ Pasteur pipettes to transfer serum to slides. Still, make sure you don't wash away your organism. Make sure you come to check out the efforts of others as you hear someone (e.g., your instructor) shout Eureka! Don't do "cell dimensions" on p. 437.

[41] See **Appendix 14**.

12. **eukaryote microbes** and 13. **viruses of bacteria**							
12.6.2	Thurs	6-4.226	start	plaque assay[42]	37	24-48	2
12.6.2	Thurs	5-13-161	start	starch hydrolysis[43]	37	48	4
12.6.2	Thurs	5-7.145	start	nitrate reduction test[44]**	37	24-48	4
12.6.2	Thurs	5-1.128	start	oxidation fermentation test[45]**	37	48	lab section[46]
12.6.2	Thurs	5-20.177	start	SIM medium**	37	24-48	4
12.6.2	Thurs	5-4.136	start	MRVP**	37	120	4
12.6.2	Thurs	5-8.149	start	citrate test**	37	168	4
12.6.2	Thurs	5-17.171	start	gelatinase test**	25	168	4
12.7.2	Thurs	11-1.312	start	the fungi[47]	--	--	1 or 2
12.7.2	Thurs	11-2.320	start	protozoans[48]	--	--	1 or 2
12.7.2	Thurs	11-3.327	start	helmith parasites[49]	--	--	1 or 2
weekend 6 / week 7 below							
13. **viruses of bacteria** and 14. **infectious agents**							
13.7.1	Tues	6-4.226	finish	plaque assay	--	--	2
13.7.1	Tues	5-13-161	finish	starch hydrolysis[50]	--	--	2
13.7.1	Tues	5-7.145	finish	nitrate reduction test[51]**	--	--	2
13.7.1	Tues	5-1.128	continue	oxidation fermentation test**	37	48	2

[42] See **Appendix 7**, **Appendix 8**, and **Appendix 9** for tips on diluting, pipetting, and plaquing phage, respectively.

[43] Students, please disregard this footnote.

[44] For any experiment indicated with "**" see **Appendix 11**.

[45] Inoculating for the Oxidation-Fermentation test will take some care to do correctly. Making sure that this is done correctly should be a group activity/responsibility. Note that you are instructed to heavily inoculate: "Stab several times to a depth of about one cm from the bottom of the agar." Failure to heavily inoculate will result in incomplete reactions and consequently misleading results. Also, you will be instructed to apply oil to one of each pair of inoculated tubes. Please make sure as you are doing this that you neither cross-contaminate your cultures nor contaminate the bottle containing the oil.

[46] This test will be done by the entire lab section, with results compared between lab sections if necessary (the test uses up far too much media to do the preliminary analysis on a group-by-group basis).

[47] See **Appendix 12**.

[48] See **Appendix 12**.

[49] See **Appendix 12**.

[50] You are looking for a halo around growth, not a lightness to the growth itself. See Figure 5-40 (p. 178) for an indication of what this halo should look like.

[51] After you have examined the tubes for gas ("G") you should not discard any tubes but instead run them through the test (this is because we are using more organisms, including fermenters, than the book suggests). To observe color reactions you will be adding reagents A & B to cultures. To do this, put on gloves, turn on hood (where reagents are located). Add 10 or more drops each of nitrate A and B. Vortext to mix. Watch tubes for five minutes. A red color by the end of five minutes indicates "+1" which means that the organism reduces nitrate to nitrite. If the color is not red after five minutes, add a "pinch" of zinc using a supplied spatula. Vortex. Check in 10 minutes. If the media is now red then this indicates that the organism does not reduce nitrate. Score red after zinc addition as "-". If not red then organisms reduced nitrate to something other than nitrate so score as "+2". Note that this scheme can be found on p. 163. Contamination of cultures is no longer a major concern, since you won't be using these cultures as living things any more, though you should still strive towards effective aseptic technique (e.g., don't contaminate yourself, don't contaminate your bench, don't cross-contaminate the cultures, don't do anything you don't want to get into the habit of doing, etc.). Use supplied scupulas to transfer powdered zinc. Please proceed to subsequent efforts (below) during incubations (e.g., five minutes following addition or reagents A & B and 10 minutes following addition of zinc powder).

13.7.1	Tues	5-20.177	finish	SIM medium**	--	--	2
13.7.1	Tues	11-1.312	continue	the fungi	--	--	1 or 2
13.7.1	Tues	11-2.320	continue	Protozoans	--	--	1 or 2
13.7.1	Tues	11-3.327	continue	helmith parasites	--	--	1 or 2
14. **infectious agents** and 15. **innate immunity**							
14.7.2	Thurs	5-1.128	finish	oxidation fermentation test**	--	--	2
14.7.2	Thurs	5-4.136	finish	MRVP**	--	--	2
14.7.2	Thurs	5-8.149	finish	citrate test**	--	--	2
14.7.2	Thurs	5-17.171	finish	gelatinase test[52]**	--	--	2
14.7.2	Thurs	11-1.312	continue	the fungi	--	--	1 or 2
14.7.2	Thurs	11-2.320	continue	Protozoans	--	--	1 or 2
14.7.2	Thurs	11-3.327	continue	helmith parasites	--	--	1 or 2
weekend 7 / week 8 below							
midterm exam #3							
15.8.1	Tues	5-5.141	start	catalase test[53]**	37	24	4
15.8.1	Tues	5-15.166	start	urease test (broth only)[54]**	37	24	4
15.8.1	Tues	5-16.169	start	casease test**	37	24	4
15.8.1	Tues	5-21.181	start	triple sugar iron agar**	37	18-24	4
15.8.1	Tues	5-2.131	start	phenol red broth[55]**	37	18	4
15.8.1	Tues	5-28.198	start	motility test**	37	24-48	4
15.8.1	Tues	5-23.186	start	litmus milk test**	37	168	4
16. **adaptive immunity** and 17. **immunology applications**							
16.8.2	Thurs	5-27.196	start	coagulase test[56]	37	24	2
16.8.2	Thurs	7-3.242	start	antimicrobial susceptibility[57]	37	16-18	2
16.8.2	Thurs	5-6.143	complete	oxidase test[58]**	--	--	2

[52] Students, please disregard this footnote.

[53] Make sure that you inoculate a nutrient-agar plate with each culture, four cultures per plate except *Proteus*, which goes on its own plate. **These will replace the nutrient agar slants employed for 5-5.154 and 5-6.167.**

[54] We will be doing only the broth version of the urease test.

[55] Students, please disregard this footnote.

[56] We will not be doing the slide-based test. Please follow directions, including making sure that you get your organisms from a slant rather than from a broth tube. However, a heavier, well-mixed inoculum into the tube is preferable to lighter, poorly mixed inoculum. Note that a smear does not involve huge amounts of liquid, but do put in sufficient organisms so that the smear is at least as cloudy as seen in Figure 5-80.215. Again, follow directions as written in your text—you cannot do this protocol without reading your text. Note that your text indicates that you should be looking at the tube in the incubator every half hour for at least 1.5 hours.

[57] Don't forget to tamp down on the filter-paper disks to make sure that they are fully in contact with the agar (use a flamed butt of a loop or forceps). The plates generated by the antimicrobial susceptibility exercise are to be placed on a separate tray for incubation, and incubation will be limited to 16-18 hours (18 for the first lab section, 16 for the second). The results from this test will not be viewed until after we discuss antibiotics in lecture (last lecture day of quarter), i.e., the 20.10.2 lab. Your instructor will introduce you to how to make a bacterial lawn using a cotton swab.

[58] Make sure you do the oxidase test before you do Exercise 5-5.154 (the catalase test) since you will be using the cultures grown up for the catalase test (and it will not longer be available once you do the catalase test). Oxidase test strips (or whatever we are using—it changes from year to year) and hydrogen peroxide (for the catalase test) will be put out for this lab. **Please see your instructor when you are ready to perform this test—don't do it on your own! We will try to minimize the usage of materials when performing this test (these materials are**

16.8.2	Thurs	5-5.141	finish	catalase test[59]**	--	--	2
16.8.2	Thurs	5-15.166	finish	urease test (broth only)**	--	--	2
16.8.2	Thurs	5-16.169	finish	casease test**	--	--	2
16.8.2	Thurs	5-21.181	finish	triple sugar iron agar**	--	--	2
16.8.2	Thurs	5-2.131	finish	phenol red broth**	--	--	2
16.8.2	Thurs	5-28.198	finish	motility test[60]**	--	--	2
16.8.2	Thurs	11-1.312	continue	the fungi	--	--	1 or 2
16.8.2	Thurs	11-2.320	continue	protozoans	--	--	1 or 2
16.8.2	Thurs	11-3.327	continue	helmith parasites	--	--	1 or 2
weekend 8 / week 9 below							
17. **immunology applications** and 19. **host-microbe interactions**							
17.9.1	Tues	5-26.193	start	blood agar	25	24	2
17.9.1	Tues	2-1.28	start	ubiquity of microorganisms	25,37	24-48	2
17.9.1	Tues	--	start	bacterial unknown[61]	many	many	1
17.9.1	Tues	5-4.136	start	MRVP	37	120	1
17.9.1	Tues	5-8.149	start	citrate test	37	168	1
17.9.1	Tues	5-17.171	start	gelatinase test	25	168	1
17.9.1	Tues	5-23.186	start	litmus milk test	37	168	1
17.9.1	Tues	5-27.196	finish	coagulase test	--	--	2
17.9.1	Tues	11-1.312	continue	the fungi	--	--	1 or 2
17.9.1	Tues	11-2.320	continue	protozoans	--	--	1 or 2
17.9.1	Tues	11-3.327	continue	helmith parasites	--	--	1 or 2
19. **host-microbe interactions** and 20. **epidemiology**							
18.9.2	Thurs	5-14.164	complete	ONPG test[62]	--	--	2
18.9.2	Thurs	5-26.193	finish	blood agar	--	--	2
18.9.2	Thurs	2-01.28	finish	ubiquity of microorganisms	--	--	2
18.9.2	Thurs	5-23.186	finish	litmus milk test[63]**	--	--	2

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relatively expensive). For BBL DrySlides the procedure looks like this: Employ sterile toothpicks to do the culture transfers. Spread organisms into smears of 3-4 mm in diameter. Positives will turn dark purple within 20 sec. Disregard color changes that occur after 20 sec. Disregard what your books says about applying water to slides.

[59] You will not be using a slide to do this test. Just add HOOH directly to the slant (though only after you have done your oxidase test).

[60] Make sure that you have been assembling your Chart of Differential Tests for Determination of Unknown. Note that the expectation is that each individual will assemble their own chart. This can be done in collaboration with others, but should not result in each individual sharing an electronic copy of the chart. If you need more sheets, please let your lab instructor know.

[61] Today you will be doing the Gram staining and initial inoculations for your unknown. Note that you absolutely positively need to accomplish two things today in addition to staining and inoculating differential media: (i) You need to record the number of your unknown in your lab book and (ii) you need to subculture your unknown so that you will have culture to inoculate from during the next laboratory period. See **Appendix 13**, first day, for how to proceed.

[62] Get this started early, i.e., immediately upon coming to class and before you take your quiz, because it will take approximately two hours of incubation until you can read the results.

[63] Note that litmus milk reactions can be “interesting” to interpret. Use your lab text as a guide, but do try to write down what you see (remember, your goal in these labs is to understand what it is that you are looking at). Also note that this is not your unkown litmus milk but instead the last of the tests with the panel of 13 organisms.

18.9.2	Thurs	--	continue	bacterial unknown[64]	many	many	1
18.9.2	Thurs	5-1.128	start	oxidation fermentation test	37	48	1
18.9.2	Thurs	5-2.131	start	phenol red broth	37	18	1
18.9.2	Thurs	5-5.141	start	catalase test	37	24	1
18.9.2	Thurs	5-7.145	start	nitrate reduction test	37	24-48	1
18.9.2	Thurs	5-15.166	start	urease test (broth only)	37	24	1
18.9.2	Thurs	5-16.169	start	casease test	37	24	1
18.9.2	Thurs	5-20.177	start	SIM medium	27	24-48	1
18.9.2	Thurs	5-21.181	start	triple sugar iron agar	37	18-24	1
18.9.2	Thurs	5-28.198	start	motility test	37	24-48	1
18.9.2	Thurs	11-1.312	finish	the fungi	--	--	1 or 2
18.9.2	Thurs	11-2.320	finish	protozoans	--	--	1 or 2
18.9.2	Thurs	11-3.327	finish	helmith parasites	--	--	1 or 2
weekend 9 / week 10 below							
20. **epidemiology** and 21. **antimicrobial medications**							
19.10.1	Tues	--	finish	bacterial unknown[65]	--	--	1
19.10.1	Tues	5-1.128	finish	oxidation fermentation test	--	--	1
19.10.1	Tues	5-2.131	finish	phenol red broth	--	--	1
19.10.1	Tues	5-4.136	finish	MRVP	--	--	1
19.10.1	Tues	5-6.143	finish	oxidase test	--	--	1
19.10.1	Tues	5-5.141	finish	catalase test	--	--	1
19.10.1	Tues	5-7.145	finish	nitrate reduction test	--	--	1
19.10.1	Tues	5-8.149	finish	citrate test	--	--	1
19.10.1	Tues	5-15.166	finish	urease test (broth only)	--	--	1
19.10.1	Tues	5-16.169	finish	casease test	--	--	1
19.10.1	Tues	5-17.171	finish	gelatinase test	--	--	1
19.10.1	Tues	5-20.177	finish	SIM medium	--	--	1
19.10.1	Tues	5-21.181	finish	triple sugar iron agar	--	--	1
19.10.1	Tues	5-23.186	finish	litmus milk test	--	--	1
19.10.1	Tues	5-28.198	finish	motility test	--	--	1
19.10.1	Tues	7-3.242	finish	antimicrobial susceptibility	--	--	2
21. **antimicrobial medications**							
20.10.2	Thurs	lab exam # 2	none	comprehensive[66] lab exam	--	--	1
weekend 10 / week 11 below							
review (outside of class—when do you want to do it?)							
midterm exam #4 (during final exam period)							

Appendix 1: Lab Completion

[64] See **Appendix 13**, second day, for how to proceed.
[65] See **Appendix 13**, third day, for how to proceed.[66] Techniques in particular are comprehensive. You should know, with the book's help, how to do the techniques that you have learned.

- Petri dishes must be incubated *upside* down. *Failure to turn your petri dish(es) upside down while incubating—if we catch you— will resulting in a 1-point deduction from your grade (on 1000-point scale) along with temporary confiscation of your materials.*
- You must label cultures with your name, the organism(s), the exercise, the media employed, and, to avoid confusion, sometimes the incubation temperature as well.
- The only thing that should go in regular trash are paper towels used for hand washing. Please glance at the tops of the trash bins at the end of lab periods to make sure that inappropriate materials have not been disposed in there. The goal is to protect the janitor, the garbage man, and archeologists in the future, and YOURSELF, so don't go digging through the garbage. *Presence of such materials in the garbage containers may result in a deduction of points from the clean-up portion of your and/or your entire lab-section's cleanup grade.*
- All gloves, petri dishes to be discarded, and paper towels used to clean spills all go in the large red biohazard bag container. *Presence of such materials in the garbage containers may result in a deduction of points from the clean-up portion of your and/or your entire lab-section's cleanup grade.*
- Contaminated glass, including broken slides, broken test tubes, and Pasteur pipettes all go in a sharps tub provided at each bench. *Presence of such materials in the garbage containers or the red biohazard containers may result in a deduction of points from the clean-up portion of your and/or your entire lab-section's cleanup grade.*
- Serological pipettes go in round plastic barrels present at each bench (not always supplied)
- Cotton swabs go in small biohazard bags provided at each bench.
- Red and blue "small-opening" test tube racks are returned to the front of the room, or to the same place where you obtain them from. These should be returned to a neat arrangement.
- Blue "large-opening" test tube racks are to remain on benches for NCSC use. **Do not use these racks in the incubators nor return them to the carts at the front of the room.** *We will confiscate materials that have been placed in these racks and/or reduce your overall grade by 1 point on a 1000-point scale for having done so.* If you have any questions as to what constitutes a "large-opening" NCSC rack, then please ask your lab instructor for clarification.
- Before leaving the lab, lab benches are to be cleaned and disinfected with all trash removed and any unused material returned to drawers or carts.
- All material that you put on your benches (e.g., including test-tube racks, burners, etc.) should be returned to the appropriate places and those places should be returned to a neat arrangement before you go on to the next task.
- Return stains onto carts in a neat and orderly fashion.
- To-be-discarded test tubes go in metal racks found at the front of the room, and should not be kept in plastic racks **Failure to properly dispose of test tubes in metal racks can result in an inadvertent disposal of not-to-be-discarded cultures by instructors forced to clean up after the students.** *Presence of tubes for disposal in plastic racks may result in a deduction of points from the clean-up portion of your and/or your entire lab-section's cleanup grade.* Please inform the instructor if you run out of metal racks. Note that not all caps on tubes fit tightly so be careful when transferring tubes to metal racks.
- Broken but not contaminated glassware goes directly into the broken-glass boxes. Broken and contaminated glassware goes into the tubs found in the center of benches (the tubs should have an inch or two of 10% bleach in them).
- If students have completed lab early they should devote some time to making sure that the lab is a cleaned up as possible before leaving, e.g., glance at tops of garbage cans to make sure that they do not contain any obvious sharps or biohazardous material (IF YOU SEE ANY PROBLEMS, PLEASE BRING THEM TO THE ATTENTION OF THE INSTRUCTOR), that common materials on carts or in drawers are neatly

arranged. *Do not expect that students who have not-yet completed their lab work will be able to do this work for you! Points will be deducted especially from your grade for not contributing to the team effort necessary to clean and neaten labs if you have completed your labs early!*

- When in doubt, ask the instructor for input on how to properly complete labs and otherwise clean up the laboratory environment before leaving.

Appendix 2: Microscopy.

- Please handle scopes carefully, avoid getting oil on the scopes (other than the slide and the 100x oil-immersion objective lens), clean using alcohol or lens cleaner along with cotton gauze or lens paper (if using dry), keep the condenser up as high as it will go (unless you know better), and initially close the iris diaphragm as far as it will go (opening it again only as you need more light)
- Make sure that your prepared slide is clean before you attempt to view it under the microscope. Clean as you would your optics (above).
- Making sure that you can see something on your slide with your low power lenses before continuing on to higher power is really, really important. Remember that if you move your stage and don't see any movement by what you are viewing then you have not focused in on what you ought to be viewing.
- If you have trouble finding specimens, then please call over your instructor. Do not spend tens of minutes attempting and then failing to focus your microscope. It may be that the prepared slide you are using simply does not have any specimen left on it (prepared slides can deteriorate over time). By calling over your instructor relatively early in the process you can avoid wasting a great deal of time trying to visualize something that, in fact, isn't even there.
- When you are done with the microscope, please clean it up, particularly removing oil so that it is ready for the next user. Put the microscope away only if you have no more microscopy procedures for that day or if you need to increase available bench space.
- Try to do a good job sketching what you see in the microscope; this means taking your time and making the necessary effort to make sure what you are sketching is an accurate rendition of what you are observing; you may be asked to present copies of these sketches as part of your lab exam; Check with your instructor to make sure that your sketches are reasonably accurate.
- When you are done with prepared slides, please clean them as you would your microscope when done, and return slides to the trays in which you found them.

Appendix 3: Microscopy for first lab.

- As part of an introduction to microscopy and microscopes you will need to read pages 63-68 of your lab text in class. Your laboratory instructor will provide an oral introduction to the microscope and how to both handle and use a microscope.
- Follow the directions on p. 67, "Operation," explicitly. This is how you focus a microscope. Please focus in on, view, and then carefully draw on p. 68 of your lab text the views of organisms at high and dry and under oil immersion. Note that the pedagogical goal of this procedure is for you to learn how to focus (and then to practice focusing) a microscope.
- Use the following format to record your data (i.e., put this table in the blank space on p. 68 of your lab book—the dashed numbers are for the lab setup person and can be ignored). To reduce the probability of having a difficult time reading your slide, you probably should not start with the "typical cocci" slide. You do not need to wear gloves for this procedure.

Slide	High and dry	Oil immersion

	(400x):	**(1000x):**
"typical bacillus Gram positive" (2-2)		
"spirillum" (1-3)		
"*Saccharomyces* budding (1-5)		
"blood human Wright" (1-2)		
"typical cocci" (1-1)		

- Don't forget to answer the questions associated with this exercise that are found on the blue pages in the back of your book (including table p. 429 and questions as indicated in **Appendix 15**).

Appendix 4: Smearing and Staining.

- Always wear gloves when staining since some stains are carcinogens. There are aprons that you may use found in a drawer in the back of the room. Remember that it is a good idea to not wear your best clothing on a day you will be staining.
- Use a loop to make all transfers, or whatever works for you.
- Don't forget to resuspend broth cultures prior to attempting transfers. If your smear is invisible, particularly after staining, then make sure that you are resuspending your cultures before transferring (and not just going through motions of resuspension but actually making sure that the culture is turbid when you are done—if in doubt, employ the vortexer to do this).
- Note that placing too much organism on your slide is *not* a virtue. If your smear is strongly opaque then you have transferred way too much bacteria.
- To make smears from solid cultures you will need to get a loopful of tap water. Don't forget to "carefully" turn on the water *before* placing the loop beneath it.
- Note that smears made from broth cultures do not need additional water, unlike those coming from solid media. For both, the more liquid you use, the longer it will take for the smear to dry.
- Spread your smear to about the size of a dime. Do not use vigorous spreading, however, as this can disrupt cell arrangements: be gentle in your smearing.
- Make sure that you leave enough room on the side of your slide to fix your smears without burning your fingers (or melting your gloves).
- Allowing smears to dry can take a relatively long time; to speed up this experiment, try smearing your cultures in parallel so that they are all drying at the same time. Alternatively, move on to other procedures while smears are drying.
- You can keep an eye on these smears as you return to other procedures. As your smears dry, you will proceed to stain them.
- When smears are dry, then heat fix them by passing the slide through a flame three times. Note that the slide should not get so hot that it burns you nor should it light your gloves on fire!!!
- For rinsing stain from slides use distilled water from wash bottle.
- Dispose of used slides in the tub provided. If you have not yet viewed your slides please tell your instructor so that they may be saved until the next laboratory period.

- Remember, demonstration of good smearing technique could be a practical portion of the laboratory exam, e.g., a smear of *Staphylococus aereus*, especially taken from a slant, should retain the proper arrangements and those arrangements should be found separated from other such arrangements or cells (i.e., the bright background should be abundantly visible).
- For your first lab doing staining:
    - Pair off to share organisms but do your own smearing, staining, and microscopy.
    - Don't forget to label your slides—use a red Gram-staining marker. You ***MUST*** learn to always do this. Get in the habit ***NOW***!
    - We will be looking at three organisms, *Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus epidermidis*, rather than the list of six suggested in your book.
    - For the sake of avoiding confusion why don't you stain the following organisms with following stains:
        - *B. subtilis*, round 1: Crystal Violet
        - *M. luteus*, round 1: Methylene blue
        - *S. aureus*, round 1: Safranin
        - *B. subtilis*, round 2: Safranin
        - *M. luteus*, round 2: Crystal Violet
        - *S. aureus*, round 2: Methylene blue
    - Once you have proven to yourself that you can see stained bacteria, repeat the process with three more smears, modifying your approach if necessary. That is, do each organism twice, and in serial, so that you can be critiqued on your first attempt (by yourself and your instructor) before going on to a second attempt.
    - If previously you employed too much organism you now employ less, or if previously you employed too little you now employ more.
    - If you burned your organism while heat fixing you use less heat, or if your organism was lost despite heat fixing you apply more heat.
    - If the arrangement of your *M. luteus* is simply individual cocci rather than tetrads (arrangements of four), then you apply less vigor in mixing your smear so that you don't break up the cellular arrangements.
    - If, as you continue to work on your smears, you find that you have completed your observation of your prepared slides then move on to observe the cultures you inoculated during your last lab as your smears are drying.
    - Try to implement any improvements in your technique by smearing the same three organisms a second time.
    - Please stain and observe your smears under oil immersion. Remember that one does not go directly to full magnification. Therefore, do not add oil until you have succeeded in observing your specimen using the high-and-dry objective. Do not change focus when switching objectives!
    - Don't forget that if the specimen does not move when you move the slide on the stage, then you have *not* successfully focused in on your specimen!

Appendix 5: Aseptic Transfer.

- Basic instruction on how to do aseptic transfers properly can be found on pp. 14-19 of your lab text. If you have not already then please read that material before coming to lab.

- Note on p. 18 there is no statement that you should "vigorously bang the transfer instrument against the interior of broth recipient tubes." Please do not use such technique. It potentially contaminates you, your culture, and the rest of your environment, and is an incredibly ugly habit to behold.
- To assure transfer into broth I like to scratch inoculum-holding loops (or wires) against the wall of the vessel, beneath the level of media—remember, only a single bacterium needs be transferred to succeed in inoculating a broth tube, and chances are you have millions (if not billions) of cells on your transfer instrument once you've picked up your culture.
- Make sure your cultures are in suspension before attempting to transfer from broth culture. This can be done by flicking or by vortexing cultures. Bacteria settle to the bottom of tubes, and you may not get any. Note that not resuspending cultures is a very common mistake.
- For your first lab doing aseptic transfers:
    - Try to double up (triple, quadruple, etc.) in racks when incubating if that will cut down on clutter in the incubator without creating too much work.
    - Regardless of how you share your donor (parent) cultures, make sure that you do all transfers individually (i.e., you are trying to practice these techniques, not watch another do them for you).
    - Work diligently and efficiently. If it takes you an hour to do this procedure then it may be an indication that you are not doing a terribly good job reading and studying your labs before coming to class.
- In subsequent experiments involving partners, please do not share doing your inoculations (e.g., one flaming, one holding the test tube, a third inoculating); part of the purpose of these labs is to *practice* your aseptic transfer technique!!!!!!!!!!!!!!!!!!!!!!!!!!!

Appendix 6: Quadrant Streak.

- Your instructor will demonstrate the quadrant-streak technique.
- With the quadrant streak the object is to end up with isolated colonies by successively diluting down your culture across the agar surface.
- Note that it is very possible (indeed easy) to do a really poor quadrant streak no matter what method you employ. Generally, as a rule, the more time your loop spends streaking the better your quadrant streak will come out.
- Also, a light touch when streaking is preferable, to avoid gouging the agar.
- Please practice, once you have read the directions, using a pen or pencil on a piece of paper (any sheet will do, though on p. 387 there is a practice "circle" just for doing this).

Appendix 7: Diluting.

- Because our equipment and materials are different from those suggested by the book, **we will be multiplying all dilution volumes by 10-fold.** That means that all volumes of diluent and all volumes diluted will be 10-fold greater while you are performing dilution series. In this way, all final volumes will be 10 ml rather than 1.0 ml and all volumes diluted will be either 1.0 ml or 0.1 ml (rather than 0.1 ml or 0.01 ml).

- To dilute your cultures you will need two tubes filled with 9.9 ml saline (or equivalent diluent) to which 0.1 ml of culture (or previous dilution) will be added. You will also need three tubes filled with 9.0 ml saline to which 1.0 ml previous dilution will be added.
- Can you tell, for example, from Figure 6.1 (p. 219) where these dilution tubes will be employed (i.e., given that we will be multiplying the figure-shown volumes by 10)?
- Note that we will be changing the volumes of dilutions only. We will still be placing 100 μl onto plates (using plastic micropipetters). Remember, 1 ml = 1000 μl and 100 μl = 0.1 ml.
- Remember to make sure that cultures and dilutions are well mixed (and well suspended) before removing volumes for subsequent dilutions or plating.
- Don't forget to change pipettes (or tips) after each transfer.
- Post incubation you should look at your plates to get a general idea of what plates have too many colonies (or plaques) to count, then count your colonies (or plaques).
- If you choose to employ our colony counter for this task, but it is currently being used, then take the time available to begin organizing your cultures.

Appendix 8: Pipetting.

- Handling pipettes requires some instruction, which will be provided by your instructor and which also is provided in your text (the ultimate location of lab exam questions). See pp. 349-356 for instructions on using pipettes.
- A volume of 0.1 ml is equivalent to 100μl and volumes of that magnitude may be handled employing 0.1 or 0.2 serological pipettes (which few use at all these days and which we will *not* be employing) or plastic micropipetters.
- Use a 1.0 ml serological (glass) pipette to measure 1.0 ml volumes. Do not confuse 10.0 ml pipettes with 1.0 ml pipettes.
- Take care in removing pipettes from cans or boxes so that you not only don't contaminate the pipette you are removing but also don't contaminate any of the other pipettes in the can.
- To remove serological pipettes from metal canisters do the following: Before removing top/cap off of canisters, make sure that the pipettes are extruded slightly (no less than a few centimeters)—doing this will take some practice. You can then set the canister directly on the bench or, preferable, set it either resting on its cap or flat on the bench but with the pipette end slightly off the bench. Then, carefully touching just the pipette you want to remove, lift up the pipette above the other pipettes in the canister and then pull out the pipette without touching the canister, if you can, and certainly without touching the ends of the other pipettes that stick out of the canisters.
- If you think that you have contaminated a pipette, or if you have already used the pipette (once) then place the pipette in the supplied plastic pipette tubes/barrels for subsequent cleaning.
- Touch glass pipettes only towards the top of the pipette, e.g., while seating the pipette into the pipette aid or while pulling the pipette out of the storage canisters.
- Still confused? Call over your instructor for some one on one on how to use pipettes.

Appendix 9: Plaquing Phage.

- Don't bother with the 15-minute preadsorption.
- Instead, you will add the phage dilution directly to the melted soft agar and then add three drops of indicator bacteria (which will be kept on ice). Making sure that the cap is tightly on, give the indicator a quick upright shake before using. Return indicator to ice between use. Be careful *not* to contaminate the indicator bacteria with phage, i.e., do not touch the glass dropper to phage-containing media, or anything else for that matter.
- The plating will involve mixing phage and indicator bacteria with molten "soft" agar. This agar can be found in small test tubes in a 45°C water bath. Keep in mind that this agar will solidify fairly rapidly so you should not bring it to your bench until you are immediately ready to use it.
- It may not be necessary to mix the soft agar before pouring since the pouring process can provide adequate mixing. However, you may need to manipulate the plate to make sure that this "top" agar layer fully covers the "bottom" agar layer.
- After a few seconds (<10) stop manipulating the plate and let it sit for a minute or two so that the soft/top agar can solidify before you invert the plate for incubation.

Appendix 10: Use-Dilution Test.

- This may be the most time-consuming thing you do on this day so make sure that you work efficiently through your other exercises so that you can get started on it relatively early.
- The procedure will involve coating ceramic beads with bacteria and then placing those beads in various dilutions of disinfectants. Your instructor will introduce to you how to do this.
- We will be doing this procedure as an entire lab, with different groups assigned, by your instructor, to different organism-disinfectant-dilution combinations. If we have 24 students in a lab then we can break up into pairs with each pair doing one bacterial species-disinfectant dilution series. If we have less than 24 students then we will need to come up with a different combination. For example, we can have four groups where each group is responsible for one disinfectant (all three dilutions) and all three bacterial species.
- Note that you should use a new sterile petri dish and new solutions of disinfectant and wash water for each organism.
- Note that in the Fig. 2-14.59 you be doing the following:
  1. Using alcohol-flamed forceps remove four sterile beads of the same color and place those beads in a tube containing a given culture. Use the following conventions: red beads for *S. aureus*, blue beads for *B. cereus*, and yellow beads for *M. smegmatis*.
  2. After 1 minute decant the broth and beads into a sterile petri dish. Note that you can use both bottoms and tops for this step since there will be no need to place a cover on the petri dish following this decanting. Your goal will be to have beads in the petri dish which you can reach using your alcohol-flamed forceps.
  3. Again using alcohol-flamed forceps, remove all four beads to a petri dish containing filter paper and allow the culture to dry onto the beads.
  4. Three of these four beads are now transferred to three places: (top red) a petri dish containing the least-diluted disinfectant, (middle red) a petri dish containing the next-diluted disinfectant, and (lower red) a petri dish containing the most-diluted disinfectant.
  5. After 10 minutes the beads from the red dishes should be transferred to dishes containing sterile water.
  6. The fourth bead from step 4 should also be transferred to sterile water (this is your growth-positive control).
  7. Transfer all four beads to separate sterile nutrient-broth blanks.

8. Transfer a sterile bead to sterile water for one minute and then transfer the bead to a sterile nutrient-broth blank (this is your growth-negative control).
9. When you are done with one organism you must dispose of the disinfectant down the drain and then dispose of the petri dishes to the red biohazard container that we normally dispose of petri dishes in.
10. Note that I discourage you from doing all three bacterial species simultaneously as a group endeavor. Instead, I would encourage you to break up into subgroups to test each organism as a distinct experiment.

Appendix 11: Differential Tests and Panel of Possible Unknowns.

- Your job is to learn how to use these tests. Therefore you must observe how each (or, at least, the more uniquely inoculated) test is inoculated.
- To be effective inoculators as a group you will need to figure out who is going to do what and how to share cultures—the sooner you figure this out the faster this and subsequent labs will go.
- You will need to put forth an effort towards properly labeling and properly incubating plates.
- You must avoid cross-contaminating cultures and you must go out of your way to make sure that culture tubes are marked correctly. Do not return cultures to where you obtained them. In fact, rather than assigning one individual to a given test, assign each individual to a given organism. Therefore the likelihood of cross contamination will be reduced, the each individual will have the opportunity to work with each test, and nobody will be able to shirk their inoculating responsibilities to the rest of the group. (work it out among yourselves about who will do the inoculating of odd-out organisms, e.g., the 13th—perhaps you can divvy up by test for just that one)
- You must follow directions in your book and on this sheet for how best to perform these tests, both in terms of inoculating media and in terms of observing cultures during subsequent lab periods.
- You must label your media, minimally in terms of your group's name, what media is being used, and at least some code as to what organism is being used. For example, you can number the tubes 1-14 in alphabetical order as presented above (with 14 the control).
- Do not write over media on tubes (since you may not be able to read your writing if the media changes color). And do not use a Gram-staining pen to mark tubes.
- The panel of organisms you will be using for many of these tests (those marked with "**") are listed in **Appendix 13**. You will be inoculating using all of the following organisms.
- for inoculations of petri dishes employ spot inoculations of four organisms per plate for three plates, and then employ the fourth plate for a spot inoculation with *Proteus vulgaris* only.
- Make sure you also incubate a not-inoculated negative control for each medium.
- Try to double up (triple, quadruple, etc.) in racks when incubating if that will cut down on clutter in the incubator without creating too much work.
- Your goal, post incubation, is to accurately record the results for the various tests. It is permissible to compare your results with those of other groups, or to call over your instructor for their opinion. However, the better job that you can do distinguishing the reactions associated with different organisms, the easier it will be for you to identify your unknown. It may be that some results are ambiguous, just make sure that you write down what you see, rather than what you think you ought to be seeing, the more detail the better.
- It will be much easier to assess your results if you take the time to separate out your cultures by experiment and then, within experiments, place all of your cultures linearly in order (e.g., alphabetically by organism), and then observe them in order. For example, when you are observing tubes, line up all 14 tubes, in order (i.e., 1-13 in the order listed in **Appendix 13**) and then simply observe your tubes going from left to right. This approach will greatly increase the efficiency with which you and your group can view the materials.

- If you find that observations are "taking forever" then it is likely either that you have not come to class prepared for what you will be looking for in a given experiment (i.e., by reading ahead—don't forget that you may be quizzed on what you should be looking for) and/or that you have not organized your materials to make observations of cultures fast and easy. To assist in this organization, please ask your instructor if you will need additional test tube racks but can't find any (with luck we will have more in the prep lab).
- Observe cultures as a group, or at least sequentially through a group. Everybody needs to see the reactions. To bring home this point, note that though you inoculated as a group of four, our expectation is that you will observe as a group of two.
- Make sure that you record what you see, not what you think you should see, including making note of subtle differences among bacterial species. And feel free to compare your results with those of your neighbors, consult Bergey's, and otherwise ask questions.
- As you go through these results you will assemble a chart of differential test results in order to determine your bacterial unknown (which will be one of these organisms). See **Appendix 16** for a version of what this chart should look like, on a per-text basis.

Appendix 12: Viewing slide of Eukaryotic Microbes.

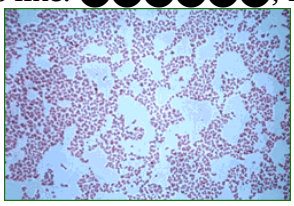
- By all means do this exercise by yourself if you would like to play with the microscope as well as take your time looking at the specimens.
- If you do this in pairs please make sure that there is approximately equal sharing in using the microscope—part of this exercise is yet additional practice in using the microscope.
- Use the following strategy: Use the photos in the text to get an idea both of what the organisms should look like and what magnification you should try to look at the organisms with. That is, if the organisms is seen under 50x in your book then you probably shouldn't try examining it at 1000x. Also note that all magnifications in the book are for ocular times object lenses, not just for objective lens alone.
- Some of these organisms can be quite difficult to find, particularly since many of these fecal samples have a great deal of debris in addition to the organisms. Also, complicating your search, these are not fresh organisms while the photos in your text are of fresh organisms—do employ the photos in the text to help in your search for the organisms in question.
- We have the following slides or organisms, all of which you should take a look at:
    - 10-1: *Candida albicans*, smear (A-157a)
    - 10-1: *Penicillium* conidia, w.m. (4-10a)
    - 10-1: *Saccharomyces cerevisiae* (living culture, simple stain)
    - 10-1: *Aspergillus niger* (living culture, **instructor will indicate how/if to stain**)
    - 10-1: *Penicillium* spp. (or other living culture, **ditto**)
    - 10-1: *Rhizopus* spp. (or other living culture, **ditto**)
    - 10-2: *Entamoeba coli* trophozoites, smear (ZA 1-341)
    - 10-2: *Entamoeba histolytica* trophozoite smear (ZA 1-321)
    - 10-2: *Entamoeba histolytica*, cysts: smear (ZA 1-322)
    - 10-2: *Entomoeba coli*, cysts, smear (ZA 1-342)
    - 10-2: *Giardia lamblia* trophozoites, smear (ZA 2-621)
    - 10-2: *Giardia lamblia*, cysts: smear (ZA 2-622)
    - 10-2: *Toxoplasma gondii* smear (92 W 4836)
    - 10-2: *Trichomonas vaginalis* smear (92 W 4273)
    - 10-3: *Ascaris lumbricoides* eggs w.m. (92 W 5673)

  - 10-3: *Echinococcus granulosus* hydatid cyst sec (92 W 5322)
  - 10-3: Hookworm eggs w.m. (92 W 5727)
  - 10-3: *Strongyliodes* larve w.m. (92 W 5744)
- We may have additional organisms, to be announced.
- Note that there may be limits to how much magnification may be useful for observing especially the larger of these creatures
- Please be nice to these slides, including cleaning off any oil and returning them to their rack when you are done.
- Fill in blanks on p. 605 through 618 as appropriate, with blanks filled in with any organisms that you may have looked at but which are not otherwise indicated (remembering to change the associated label). Please try to draw these organisms well. One approach to testing I may employ is to place slides on the microscope and ask you to ID them, which you will be able to do based on how well you sketched them in your lab text.

Appendix 13: Bacterial Unknown.

- Your bacterial unknown will consist of one of these organisms:
  1. *Alcaligenes faecalis*
  2. *Bacillus cereus*
  3. *Corynebacterium xerosis*
  4. *Enterobacter aerogenes*
  5. *Escherichia coli*
  6. *Klebsiella pneumoniae*
  7. *Micrococcus luteus*
  8. *Proteus vulgaris*
  9. *Pseudomonas aeruginosa*
  10. *Salmonella typhimurium*
  11. *Shigella flexneri*
  12. *Staphylococcus aureus*
  13. *Streptococcus lactis*
- Day 1 (start):
  - Don’t forget to write down the number of your unknown.
  - Long incubations should be started this day: 5-4.136, 5-8.149, 5-17.171, 5-23.186.
  - Note that none of the cultures you inoculate today will be looked at until one week from now.
  - Your unknown should be subcultured on a slant and in broth for use during next laboratory period. The two different media are so that you can inoculate from either a slant or from both, depending on preference or circumstances. During the next laboratory period you will only use your subcultures to inoculate new cultures, as directed.
  - Make sure that you do your Gram stain today.

- Note, this is what a coccus looks like: ●; this is what a diplococcus looks like: ●●; this is what a streptococcus looks like: ●●●●●●; note that both of the following are images of rods:

- Your instructor will provide a test tube rack for your unknown stock tube. This tube will be kept in this rack under refrigeration until you have completed identifying your unknown.

- Day 2 (continue):
  - Today we will *not* be looking at the unknown cultures that you inoculated during the last laboratory period.
  - Short incubations should be started today (5-1.128, 5-2.131, 5-5.141, 5-7.145, 5-15.166-broth only, 5-20.177, 5-28.198).
- Day 3 (finish):
  - You are absolutely positively not allowed to talk to other students about your or their unknowns during this period, or to share data, or to borrow data, or to collaborate in any way. Failure to follow these rules will result in forfeiture of your unknown points.
  - Please call over your instructor when you think you know what your unknown is.
  - Make sure that you have a relatively neat collection of the sheets of paper you recorded your data on.

Appendix 14: Lab Exams.

- The lab exam is open lab text and covers all of the exercises, readings, techniques, and questions assigned up to this point in the lab portion of this course. To do well you should review your lab text (well) prior to taking the lab exam. In the exam you will be tested on lab results, but *in particular you will be tested on what results mean*. Do not speak to one another during the exam.
- You will be expected to complete the lab exam within a single two-hour laboratory period. To do this you will need to come to the lab exam prepared to take the exam. That is, you should be sufficiently familiar with your lab text, the material covered, and the techniques involved to allow yourself to answer questions and perform techniques relatively rapidly. You should also come to lab with assigned questions (blue pages) completed.
- As part of your lab-exam grade you will turn in your lab text along with your exam. Your lab text will be subsequently returned to you. Make sure that you have your name somewhere in/on your lab text so that we can tell which one is yours.
- We will invoke some sort of exam scheduling scheme to make sure that all students will be in the lab room at one time, if only briefly, during administration of the exam.
- For more on laboratory exams, see www.phage.org/school_syllabus.htm#laboratory_exams, i.e., the discussion of laboratory exams as presented in your syllabus.

Appendix 15: Blue-Page Questions.

Exercise:	White pages:	Blue pages:	Questions to do:	Questions to ignore:	Exam	I've answered ?s:
1-2	14	385-386	none	1, 2, 3	1	
1-3	20	387-388	1, 2, 3, 4, 5, 6	none	1	☐
1-4	23	389-390	3, 4, 5	1, 2	1	☐
2-1	28	391-394	1, 4, 5, 6	2, 3	2	☐
2-5	40	401-402	1, 2, 3, 4, 5	none	1	☐
2-6	42	403-404	1, 2, 5	3, 4	1	☐
2-7	44	405-406	none	1, 2, 3	1	
2-10	49	411-412	2, 5	1, 3, 4	1	☐
2-14	58	425-426	2, 3, 4	1	1	☐
2-15	61	427-428	3	1, 2, 4	1	☐
3-1	64	429-430	1, 2, 5, 6, 7	3, 4	1	☐
3-4	81	435-436	2	1, 3, 4	1	☐
3-5	84	437-438	1	2, 3	1	☐
3-6	86	439-440	1, 2, 3	none	1	☐
3-7	91	441-442	1, 2, 3	none	1	☐
3-8	95	443-444	1, 2	3	1	☐
3-9	97	445-446	1, 2, 3, 4	none	1	
4-1	108	453-454	1, 2, 3, 4	5	1	☐
4-2	111	455-456	4, 5	1, 2, 3	1	
4-4	116	459-460	2, 3, 4	1	1	☐
4-5	118	461-462	5, 6, 7	1, 2, 3, 4	1	
4-7	122	465-466	2, 3, 5	1, 4	1	☐
5-1	128	469-470	none	1, 2, 3, 4, 5	2	
5-2	131	471-472	1, 3	2, 4	2	☐
5-4	136	475-476	none	1, 2, 3, 4, 5	2	
5-5	141	477-478	none	1, 2, 3, 4, 5, 6	2	
5-6	143	479-480	1, 2	3	2	☐
5-7	145	481-482	1, 4	2, 3	2	☐
5-8	149	483-484	1[67], 3	2	2	☐
5-13	161	493-494	1, 2, 3	4	2	☐
5-14	164	495-496	1, 3, 4	2	2	☐
5-15	166	497-498	1, 2	3, 4	2	☐
5-16	169	499-500	1, 2, 3, 4, 5	none	2	☐
5-17	171	501-502	1, 2, 3	4, 5	2	☐
5-20	177	507-508	2	1, 3, 4	2	☐
5-21	181	509-510	1, 2, 3	none	2	☐
5-23	186	513-514	2	1, 3	2	☐
5-26	193	519-520	2, 3	1	2	☐
5-27	196	521-522	none	1, 2, 3, 4	2	
5-28	198	523-524	1	2, 3, 4	2	☐
6-1	218	533-536	lots ☺	☺	1	☐
6-3	224	539-540	1, 2	3, 4	1	☐
6-4	226	541-542	1, 4, 5	2, 3	2	☐
7-3	242	559-560	3, 4	1, 2	2	☐
9-1	282	587-588	1, 2	3	1	☐

[67] Ignore "Refer to Appendix B for help." Doing so will just confuse the issue.

Appendix 16: Sample Chart for Differential-Test Results.

What is your name? _____

What experiment is this? _____ What is today's date? _____

organism	observation #1	observation #2	interpretation	code
1. *Alcaligenes faecalis*				
2. *Bacillus cereus*				
3. *Corynebacterium xerosis*				
4. *Eneterobacter aerogenes*				
5. *Escherichia coli*				
6. *Klebsiella pneumoniae*				
7. *Micrococcus luteus*				
8. *Pseudomonas aeruginosa*				
9. *Proteus vulgaris*				
10. *Salmonella typhimurium*				
11. *Shigella flexneri*				
12. *Staphylococcus aureus*				
13. *Streptococcus lactis*				
14. Negative Control				