Microbiology Prep Handbook

Introduction

This booklet has been written to supplement the lab manual, *Microbiology: Experiments and Lab Techniques.* It is a compilation of procedures, sources, recipes, notes, and "aha's" that we have accumulated over the years while preparing and using these lab exercises. Although we have tried to be as complete as possible without becoming tedious, there are no doubt some assumptions or details that we have overlooked. Therefore, if you have any questions or need any further help, don't hesitate to contact us

The description for each exercise begins with some brief notes to the instructor. The length of the lab is included, although this time span is quite variable depending on the amount of introductory discussion, use of quizzes or other graded activities, and of course the students themselves. In addition, we have inserted specific notes that we hope might be helpful for particular labs. Following this lab prep manual, we have attached a set of answers to the discussion questions of the student's lab manual. However, if any of your students have the audacity to come up with answers that are better than ours, you should probably give them credit anyway.

The body of each description focuses on lab preparation. Sources of materials are in most cases simply examples with many substitutions available. However, sometimes seeing an actual catalog number can save a great deal of time and frustration. Rather than repeat recipes and notes in various labs, we have added an index that refers to page of the initial discussion of that medium, reagent or technique. Arranged just prior to the index, we have also included a list of stock cultures with notes on their dietary preferences and environmental yearnings.

This set of "Lab Preparation Notes" is constantly changing and evolving as we learn better and more efficient ways of doing things. Whenever improvements develop we will continue to share them with you. Likewise, if you have any discoveries, suggestions, corrections, or "secrets" that you would like to share with fellow instructors and lab technicians, please let us know and we will pass them along. Perhaps we can eventually develop a support network (or support group?) together.

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Exercise 1: Contamination

Instructor's Notes: ~ 15 minutes. We usually combine this lab with "Exercise 2" for the first lab period.

- I use the additional time for introductory lecture and going over safety regulations (Appendix A). I also use some extra lab time to administer a non-graded pre-test to check students' knowledge of prerequisite information. Although they don't usually do that well, many of them become motivated to review. Also while they are testing, I use a seating chart to learn their names.

Safety Considerations:

- There is no way of knowing ahead of time what species of microbes might be cultured from the environment. When students are observing their colonies, we have them keep their plates closed. Incubating the plates at room temperature would also decrease the growth of many human pathogens.

Materials per student:

1. Tryptic Soy Agar (TSA)
2. Nutrient Agar plate (NA)
3. Sabouraud Dextrose plate (SAB)

Note: To make good, dry plates, the media should be poured as soon as it is removed from the autoclave into the plates arranged in stacks of four. (We pour about 20 ml per plate or 30 plates per 600 ml flask of agar.) When plates are solid, the condensation on the top plates of each stack should be removed by quickly flicking the lid. The plates will be ready for use in 48 hrs and will remain fresh for 1 week. All plates

should be labeled.

4. Test tubes with 3 sterile swabs each (Place cotton swabs, tip down, in a test tube, cap the tube, and autoclave on dry cycle.)

   Note: ☛ Cotton swabs, also called applicators, are available from CMS #325-571.

   ☛ We use slip top caps for easy placement and removal (Bacti-capalls from Fisher #14-127-28C). Bacti-capalls are technically considered "disposable" by their manufacturer, but for classroom purposes we have successfully used them until they wear out and no longer stay on the tube.

Check:

1. If this is the first lab of the semester, check to see that the incubators are on & set for 37^{o}C.

2. Masking tape is in dispenser

3. Disinfectant is filled up.

   Note: We use Sanisol Plus Disinfectant (Hardy Diagnostic's 57184)

Exercise 2: Handwashing

Instructor's Notes: ~ 30 minutes. We usually combine this lab with "Exercise 1" for the first lab period.

- This exercise can be used as a spring board for a discussion on the experimental method and the scientific approach to answering questions. If there is insufficient time for students to design their own experiments, an open ended experiment can be provided, like having students compare air-drying their hands vs. towel drying. Will wet hands pick up more contaminants from a non-sterile paper towel or from extended drying time in the air. The outcome of this experiment is not that easy for students to predict (answer: air).
- Typically students will actually find an increase in bacterial counts from washed hands vs. unwashed hands. The surfactant action of soap loosens microbes and allows higher numbers to end up on the agar plate. Therefore in this experiment we are analyzing the effectiveness of handwashing by checking the number of kinds of colonies rather than total number.
- In a second lab you can discuss the scientific process in more detail. It is also possible to follow up by allowing students to design and carry out modifications to their original experiment. This can provide a more realistic simulation of the scientific process of hypothesis formation, experimentation, revision of hypothesis and further experimentation.

Safety Considerations:

- There is always a possibility of culturing pathogenic or opportunistic microbes from human skin. When students are observing their colonies, we have them keep their plates closed. Incubating the plates at room temperature would also decrease the growth of many human pathogens.

Lab Prep Notes Exercise 2: Handwashing

Materials per student:

1. TSA (Tryptic Soy Agar) (or any enriched agar plate)

2. Hand soap, next to sink
   (We use Vionex Handsoap, Hardy Diagnostics' #25158)

3. Additional kinds of soap for possible student experiments.
   (e.g. Ivory is good because it is not antibacterial)

4. Other materials that could be provided for students to experiment with include thermometers, scrub brushes, cloth towels, hair (hand) dryers, etc.

Exercise 3: Introduction to the Microscope

Instructor's Notes: ~ 1 hour. We usually combine this lab with "Exercise 4". In addition, I spend a good deal of time discussing the use and care of the microscope.

Materials for class:

1. Extra lens paper
2. Several pairs of scissors
3. Clean slides and coverslips

Check:

1. All microscopes work & lenses are clean.

Exercise 4: Measuring Microorganisms

Instructor's Notes: ⏲ ~ 1.5 hour. We usually combine this lab with "Exercise 3".

☛ Because this lab involves measuring, I use this opportunity to introduce the metric system to students and refer them to "Appendix B". Students have had very good success learning how to make metric conversions using the technique described in this appendix.

Materials for class:

1. Several stage micrometers (the more the better)
   Note: The micrometers should all be of the same type in order to make instructions easier. The procedure described in the lab manual is based on a stage micrometer with white lines to contrast with the black lines of the ocular micrometer in the microscope. (It is produced by Leica (#31-16-90) and is available from Carolina Biological (#K3-59-4480).

2. Prepared clean slides:

   Taenia pisiformis composite w.m. (tapeworm) (Ward's #92W5400 or Carolina PS1800)
   Note: The slides needed are the ones that have four sections of tapeworm (scolex, immature, mature and gravid proglottids) on each slide.

   Trypanosoma blood smear (Ward's #92W4350)

Exercise 5: Advanced Microscopy

Instructor's Notes: ⏲ 2-3 hours. The time is quite variable depending on how much effort is devoted to exploring the pond water. I also usually spend some time discussing the different types of microscopy.

☛ Most of the protozoa cultures will contain other species in addition to the organism on the label. Therefore I give students a brief oral description (and a crude drawing on the board) of the organisms we are searching for.

Cultures:

Euglena
Amoeba
Paramecium
Pond water - pond or aquarium water. I am lucky enough to have a pond in my backyard that is loaded with interesting critters. If your source of pond water is not so diverse, you can "enrich" it with added species such as *Volvox*, *Blepharisma*, *Vorticella*, Nematodes, *Anabena*. Carolina Biological sells a pond mixture (F6-L25B) or mixed ciliates (F6-L52F) or mixed flagellates (F6-L52B).

Note: Each culture should have at least two labeled Pasteur pipets with latex bulbs (VWR's #56311-049).

Materials for class:

1. Depression slides, coverslips and regular clean slides

2. Silicone culture gum
   Note: Silicone culture gum (Ward's #37W9810) is reusable. Used culture gum is simply replaced in its original container. We clean and disinfect ours by placing it in a beaker of boiling water for 20 minutes (as per Ward's instructions).

3. Slowing agent such as Protoslo (methyl cellulose) (Ward's #37W7951)

4. Protozoan identification keys (e.g. *How to Know the Protozoa*, from W.C. Brown)

5. Tray with disinfectant for depression slides

Exercise 6: Transfer Technique

Instructor's Notes: ⏲ ~ 1.5 hour.

- ☛ I introduce the lab by discussing and showing examples of different categories of media and discussing the importance of aseptic technique in medicine and experimental research. I also perform a demonstration of one or two aseptic transfers.
- ☛ The painting exercise is meant to be a fun way to get practice making transfers. The more colored cultures available the better. Students may have some pigmented species that they saved from the contamination lab, and I encourage them to share these with one another.

Cultures: **Note:** In general, whenever we are using bacterial cultures, we provide one set of cultures for each 3-4 students to share. Unless specified otherwise we use tryptic soy broth for the broth cultures and tryptic soy agar (TSA) for the slants and plates.

Serratia marcescens	Broths
Micrococcus luteus	Slants
Bacillus cereus	Plates

Five plates of pigmented organisms - as many different colors as possible. These plates are used to draw pictures. You will need to provide one complete set for each section. Suggested organisms include: *Micrococcus luteus* (yellow), *Serratia marcescens* (red), *Chromobacterium violaceum* (purple), *Sarcina aurantiaca* (orange), also include something like *E. coli* to provide white. (All available from Ward's and Carolina)

Materials per student:

1. 3 nutrient agar slants
2. 6-8 nutrient broths
3. 1 nutrient agar plate

Teaching Demo: display jars of dehydrated media - nutrient broth, agar and EMB agar. Teacher can use these visual aids to assist in discussion of types of media.

Exercise 14: Aseptic Technique

Instructor's Notes: ⏲ 1 hour. We combine this lab with "Exercise 13".

Plate culture:

Serratia marcescens

Materials per student:

1. 1 TSA plate

2. 1 large test tube

3. 1 package of sterile gloves (VWR's #32918-257)
   Note: 1-6% of the general population and 6-17% of health care workers are allergic to natural rubber latex. Latex allergies can be severe including cardiac arrest. Question students and provide alternatives for allergic students. Unfortunately, synthetic and powder free gloves don't fit as well and are much more expensive.

4. 1 sterile package containing a glass tube
   Note: Use a piece of glass tubing that will fit into the test tube (item 2 above). Wrap the tube in a paper towel and seal with autoclave tape. Autoclave on dry cycle.

Exercise 15: Control of Microorganisms: Moist & Dry Heat

Instructor's Notes:
- 1 hour.
- ☛ Some semesters we do all the "Control" exercises (Ex. 15-18) on the same day. There really is enough time for them to complete all four exercises in a three-hour lab period if they remain organized.
- ☛ This exercise provides students with some additional practice with streak plates.
- ☛ For the demonstration one test tube of egg albumin is placed into boiling water, while the other is placed into the dry oven. I have students watch the clock, and we compare how long it takes for moist heat vs. dry heat to coagulate protein. Moist heat is always much faster because water transfers heat more effectively than dry air. The contrast is even more dramatic if you add a small amount of water to the test tube going into the water bath.

Note: If multiple exercises are combined for one lab period, it helps students considerably to have all the materials for each exercise located together in a labeled area (e.g. "Heat Experiment").

Slant cultures:

Micrococcus luteus
Bacillus subtilis (4 day old culture to insure spores)

Materials per student:

1. 1 nutrient broth

2. 4 nutrient agar plates
   Note: If students started the "Specimen Handling" exercise in the previous lab, remember to set out an additional 2 plates per student for them to continue with that experiment.

Lab Prep Notes — Exercise 15: Control by Heat

Additional materials for class:

1. Several hot plates with beakers of boiling water
   Note: We use about 5 set-ups for a class of 30.

2. 1 hot plate with beaker of boiling water in front of room for demonstration
   Note: One of the student set-ups can be used for the demo instead, if it is near the front of the room.

3. 1 oven at 100^{o}C in front of room for demonstration
   Note: We use a donated toaster oven.

4. An equal amount of egg white (albumin) dispensed into two large test tubes in front of room for demonstration

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