Miramar College, Biology 205 Microbiology
Lab Exam II Study Guide

In addition to this study guide, use your notes, text, lab manual and other resources (*i.e.*, **the *Objectives* & *Discussion* section of the Labs and the bold words in the *Background* and *Introduction* sections**) to make sure that you are fully prepared for your exam. Topics & experiments covered in lab are fair game, even if you personally did not perform them.

Lab 9: Using the Spectrophotometer and Micropipetter

- Define: serial dilution, quantitative, qualitative, indirect, direct, blank, zeroing, spectrophotometer, micropipetter.
- Draw out the serial dilution of dye in water to 10^{-5} in 3ml total volume. Remember that 1000µl = 1ml; and 10^{-1} = 0.1 = 1/10, so 1/10 of the total volume should be the dye.
- Understand the math behind preparing a 10^{-1} dilution and a 10^{-2} dilution.
- Be able to evaluate proper serial dilution based on OD readings.

Lab 10: Bacterial Growth Curve & Serial Dilutions

- Define: population growth curve, generation time, lag phase, exponential phase, stationary phase, death phase, optical density, indirect, direct, quantitative, colony forming unit.
- Draw out the serial dilution of bacteria in water to 10^{-7} in 10ml total volume. Remember that 1000µl = 1ml; 10^{-1} = 0.1 = 1/10, so 1/10 of the total volume should be the bacterial culture; and 10^{-2} = 0.01 = 1/100, so 1/100 of the total volume should be the bacterial culture.
- From these dilutions, calculate original CFUs/ml. Why are these numbers expressed as CFUs/ml and not cells/ml? What is the statistically significant range of CFUs on a plate?
- Understand the differences between direct & indirect measurements of growth. Why are spectrophotometer readings considered indirect, while dilutions and plate counts are direct?
- Understand why it is necessary to dilute the culture prior to spread plating.
- Be able to graph OD data and determine the generation time of the culture.

Lab 11: Physical Growth Requirements

- Define: toxic/reactive oxygen species, differential medium, FTM, reducing agent, thioglycollate, redox indicator, resazurin, acid, base, alkaline, (strict-obligate) anaerobe, (strict/obligate) aerobe, microaerophile, facultative anaerobe, aerotolerant anaerobe, acidophile, alkalinophile, neutrophile, mesophile, psychrophile, thermophile, hyperthermophile, psychrotroph.
- How do oxygen, temperature, and pH affect microbial growth? Are extremes in these environments bacteriostatic, bactericidal or bacteriolytic?
- Describe organisms who thrive in various oxygen, temperature, and pH using scientific terminology (*e.g.*, acidophile, facultative anaerobe, etc.) based on their growth in this lab.
- Be able to graph pH or temperature data correctly to show the optimum of a given bacterial species.
- Be able to recognize the oxygen requirement of an organism grown in FTM.
- Understand how FTM and anaerobic jars provide an oxygen free environment.
- Know the chemical make-up of FTM. Why are resazurin, thioglycollate, and agar added?

Lab 12: Physical Growth Control

- Define: sterilization, sanitization, heat-labile, -static, -cidal, -lytic, non-ionizing radiation, pyrimidine/thymine dimer, mutation, thermal death time, thermal death point.
- How specifically do temperature and UV control microbial growth?
- Why are control plates necessary for these experiments?
- Understand how to interpret data and determine TDP and TDT.
- Do spores increase the survivability of organisms exposed to these physical methods of control?

Lab 13: Chemical Growth Control

- Define: resident microbes/normal flora/normal microbiota, transient microbes, antiseptic, disinfectant, disk diffusion method; seeding,
- For what kind of microbial control do disk diffusion methods test?
- Measure zone of inhibition and determine whether a given bacterial species is sensitive to a given antibiotic and/or disinfectant.
- How did Gram negatives, Gram positives, and spore-formers survive in the presence of chemical agents?

Lab 14: pAra Transformation & Appendix I

- Define: horizontal gene transfer, transformation, donor cell, recipient cell, recombinant cell, vector, heat-shock, promoter, selective marker, origin of replication, activator sequence, reporter gene.
- Understand the overall function of the arabinose operon, in regard to its use in this experiment.
- What are the five components of plasmids, and the pAra plasmid specifically?
- What is the importance of each of the following: competent cells, $CaCl_2$, heat shock, ampicillin, arabinose, *araC*, *rfp*, Red Fluorescent Protein, and *pBAD*?
- What did you expect to grow on each of the plates (*e.g.*, LB, LB/amp, LB/Amp/Ara)? Discuss for both P+ and P- tubes.
- How are successful transformants identified?

Lab 15 & 16: DNA Fingerprinting & Appendix II

- Define: DNA fingerprint, PCR, amplify, denaturing DNA, annealing DNA, elongating DNA, primer, dNTP, DNA polymerase, restriction enzyme, digestion, restriction site, restriction fragment, RFLP, agarose gel electrophoresis, loading dye.
- Why is each of the following components of PCR necessary: primers, thermophilic DNA polymerase, buffer, dNTPs, thermocycler)
- What happens during the denaturing, annealing, and elongation steps of PCR- include the temperature of each.
- Understand how restriction digests work- including restriction site and sticky ends. Including the necessary components (*e.g.*, restriction enzyme, buffer).
- What happens during electrophoresis? Why is an electrical field and an agarose matrix necessary? Why do molecules of DNA move at different rates? What makes them move at all?
- Why is loading dye added to samples?
- What is a DNA fingerprint? How is an RFLP DNA fingerprint made?
- Identify identical DNA fingerprints on an agarose gel.

Lab 17: Therapeutic Phage Typing of *E. coli*

- Define bacteriophage, plaque, PFU and host cell. What do bacteriophage infect?
- What is phage therapy?
- Be able to set up a series of bacteriophage dilutions to get a plate at a given dilution (*e.g.*, 10^{-7} plate). Include the volume of each culture transfer, the volume of water in which they are diluted, and the amount of each dilution plated. From these dilutions, calculate original PFUs/ml. What is the statistically significant range of PFUs on a plate?
- Why do these dilutions need to have *E. coli* added to them prior to plating?
- Understand why it is necessary to dilute the bacteriophage culture prior to spread plating and counting.

LE 18: Examining Water Quality: Most Probable Number & Colilert

- Understand what is being measured by MPN and Colilert® reagents.
- Why are ONPG and MUG both included in the Colilert Test Kit?
- Differentiate between a presumptive water quality test and confirmed test.
- Why are *E. coli* considered an indicator organism?
- Determine MPN using an MPN table, when shown the results of an MPN series inoculation.

Lab 19: Data Collection & Analysis

- Define: discrete data, continuous data.
- Understand the difference between discrete and continuous data, and when each would be used for graphing data.
- Be able to make a graph using discrete data and continuous data.
- Understand how to present data clearly, and how to interpret data that have been graphed.