

MICROBIOLOGY FUNDAMENTALS LAB MANUAL



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Microbiology Fundamentals Lab Manual

By: Heather Deter, PhD

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TABLE OF CONTENTS

Title Page

- 1.1: About the Author

Licensing

1: Biosafety Practices and Procedures for the Microbiology Laboratory

2: Laboratory Methods

- 2.1: The Scientific Method
- 2.2: The Metric System and Measurement
- 2.3: Reading- Descriptive Statistics and Graphing
- 2.4: Keeping a Lab Notebook

3: Introduction to Microscopy and Diversity of Cell Types

- 3.1: Introduction
- 3.2: Procedures
- 3.3: Results
- 3.4: Review Questions
- 3.5: Scientific Nomenclature

4: Introduction to Aseptic Techniques and Growth Media

- 4.1: Introduction
- 4.2: Procedure
- 4.3: Results
- 4.4: Review Questions
- 4.5: Examples of Bacterial Growth Characteristics in Broths, Slants and Plates

5: Quantifying bacterial cultures

- 5.1: Determination of Bacterial Numbers
- 5.2: Introduction to Micropipetting
- 5.3: Serial Dilution
- 5.4: Procedures
- 5.5: Results
- 5.6: Dilution Worksheet and Problems

6: Preparation of Bacterial Smears and Introduction to Staining

- 6.1: Introduction
- 6.2: Procedures
- 6.3: Results
- 6.4: Summary of Common Bacterial Staining Techniques
- 6.5: Review Questions

7: Metabolic Activities of Bacteria

- 7.1: Introduction
- 7.2: Procedures
- 7.3: Results
- 7.4: Review Questions

8: DNA extraction

- 8.1: Background
- 8.2: Procedure and Review

9: Antimicrobials

- 9.1: Bacterial Susceptibility to Antibiotics (Kirby-Bauer Test)
- 9.2: Procedure
- 9.3: Results

10: Microbiology of Milk and Food

- 10.1: Introduction
- 10.2: Procedures
- 10.3: Results
- 10.4: Review Questions

11: Epidemiology

- 11.1: Epidemiology and Public Health
- 11.2: Solve the Outbreak

12: Immunology

- 12.1: Identification of White Blood Cells
- 12.2: Procedure and Results
- 12.3: Review Questions

13: Agar art

- 13.1: Introduction
- 13.2: Procedures
- 13.3: Results

[Index](#)

[Glossary](#)

[Detailed Licensing](#)

[Detailed Licensing](#)

Licensing

A detailed breakdown of this resource's licensing can be found in [Back Matter/Detailed Licensing](#).

1: Biosafety Practices and Procedures for the Microbiology Laboratory

What is Biosafety?

Microbiology is a science that investigates the biology of microscopic organisms. Although individual cells of these organisms may be directly observed with a microscope, and their shapes and activities observed, to investigate other characteristics such as metabolism or genetics, growing cells in populations (called **cultures**) is the preferred approach. For many types of experiments, all of the cells in the population must be essentially the same; such populations are called **pure cultures**. A set of techniques, mostly developed in the late 19th century by Robert Koch, Louis Pasteur, and their collaborators, permitted the isolation of bacteria from their natural environments and separation into pure cultures for further study. Probably more than anything else, these are the techniques that define microbiology as a scientific field of study.

It has been estimated that less than 1% of bacteria can be grown in culture in a laboratory. However, many types can be, and those are the ones on which we will focus. Microbiologists culture bacteria by providing them with food, water, and other growth requirements in an environment with a constant and comfortable growth temperature. These requirements vary, depending on the natural growth conditions for the microbial populations under study.

Food is provided in the **culture media** we use. Media may be in the form of a liquid (called a “broth”) or solid or semi-solid forms, either in tubes or in culture dishes (Petri plates). The choice of media depends on what you want to do or need to know about the bacteria in your cultures.

To ensure that we culture only the specific bacteria we want, and nothing else from the environment, microbiologists use a set of strict **aseptic techniques**, which protects us from the bacteria in the cultures and protects our cultures from contaminants in the environment. These methods will be demonstrated in class. In addition, specific laboratory rules must be followed for containment of microbial cultures in the laboratory, for the safety of all. For this laboratory, these practices are listed below.

Laboratory Safety Practices and Procedures

1. Remember that all bacteria are potential pathogens that may cause harm under unexpected or unusual circumstances. If you as a student have a compromised immune system or a recent extended illness, you should share those personal circumstances with your lab instructor.
2. Know where specific safety equipment is located in the laboratory, such as the fire extinguisher and the eyewash station.
3. Recognize the international symbol for biohazards and know where and how to dispose of all waste materials, particularly biohazard waste. Note that all biohazard waste must be sterilized by autoclave before it can be included in the waste stream.



4. Keep everything other than the cultures and tools you need OFF the lab bench.
5. All of the equipment and supplies used in experiments involving bacterial cultures should be sterilized. This includes the media you use and also the tools used for transferring media or bacteria, such as the inoculating instruments (loops and needles) and pipettes for liquid transfer.
6. Transfer of liquid cultures by pipette should NEVER involve suction provided by your mouth.
7. Disinfect your work area both BEFORE and AFTER working with bacterial cultures.
8. In the event of an accidental spill involving a bacterial culture, completely saturate the spill area with disinfectant, then cover with paper towels and allow the spill to sit for 10 minutes. Then, carefully remove the saturated paper towels, dispose of them in the biohazard waste, and clean the area again with disinfectant.
9. Wear gloves when working with cultures, and when your work is completed, dispose of the gloves in the biohazard garbage.
10. Long hair should be pulled back to keep it away from bacterial cultures and open flame.
11. Lab-appropriate attire must be worn, including closed-toed shoes and lab coats.

12. Make sure that lab benches are completely cleared (everything either thrown away or returned to the storage area) before you leave the lab.

Bacteria pose varying degrees of risk both in a controlled laboratory environment and in their natural settings. Therefore, the level of containment necessary for working safely with bacterial cultures also varies according to a system that classifies microbes into one of four biosafety levels (BSL), which provides minimum standards for safe handling of microbes at each level. BSLs are defined and containment practices are detailed by the Centers for Disease Control and Prevention (CDC) for laboratories in the United States. The full document, “*Biosafety in Microbiological and Biomedical Laboratories*,” can be viewed in its entirety at: <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>(opens in new window).

Prior to the first meeting of the laboratory, navigate to the following web page to review the BSL guidelines as outlined by the CDC, which includes an interactive quiz:

<http://www.cdc.gov/training/Quicklearns/biosafety/>(opens in new window)

Most of the bacterial cultures we will be working with are classified as BSL-1. Below, list three practices that should be used while you are working with BSL-1 bacteria:

1. _____
2. _____
3. _____

In a few of the labs, we will be working with bacteria that are classified as BSL-2. What additional safety practices should you employ when you work with these bacteria?

1. _____
2. _____
3. _____

After your instructor has discussed any additional safety procedures for your lab, complete the biosafety concept assessment on the following page (or one provided by your instructor) and sign the affirmation. Return the completed document to your instructor.

Microbiology Laboratory Biosafety Guidelines

Complete the following questions by writing the letter of the correct answer on the line.

- ___ 1. Biological agents are assigned to Biosafety Levels (BSLs) based on
 - a. whether they are bacteria, viruses, or other microorganisms.
 - b. their susceptibility to laboratory disinfectants.
 - c. the risk they pose to human health and the environment.
 - d. the amount of the agent that will be used in the lab.
- ___ 2. BSL 1 practices, equipment, and facilities are used when working with which type of microorganism?
 - a. Bacteria that are well characterized and not known to consistently cause disease in healthy adult humans.
 - b. Bacteria that are considered moderate-risk agents present in the community and associated with human diseases.
 - c. Indigenous or exotic bacteria with potential for aerosol transmission and associated with human disease.
 - d. Dangerous/exotic agents with a high risk of life-threatening disease.
- ___ 3. The primary hazards to people working in a BSL 1 or 2 laboratory relate to
 - a. accidental skin punctures caused by mishandling of sharp lab tools.
 - b. mucous membrane exposure due to accidental splashing of cultures.
 - c. accidental ingestion of infectious material.

d. All of the above are primary hazards in a BSL 1 or 2 lab.

___ 4. Which of the following is required when working with BSL 2 agents?

- a. Biohazard warning signage on doors and lab areas where agents are kept or used.
- b. Self-closing, double-door access to the lab facility.
- c. Clothing change when entering or leaving the lab.
- d. There are no special precautions needed for a BSL 2 lab.

___ 5. What is the appropriate disposal method for a bacterial culture grown on an agar plate?

- a. Throw the culture away in any one of the several garbage containers in the room.
- b. Throw the culture away in the garbage container marked with a biohazard sign.
- c. Allow the agar to dehydrate in the incubator and then discard in any garbage.
- d. Bacteria grown on an agar plate medium should never be disposed of.

Microbiology Laboratory Biosafety Affirmation

By signing your name below, you affirm that:

- Potential hazards in the microbiology laboratory have been identified and explained.
- You understand that the bacterial cultures used in the lab require either BSL 1 or BSL 2 containment procedures.
- Safety practices for BSL 1 and 2 have been explained and/or demonstrated to you.
- You fully understand the hazards associated with BSL 1 and 2 bacterial cultures and are willing to assume the risk.
- You are responsible for your own actions while working in a laboratory with potentially hazardous materials.

PRINT your FULL NAME _____

SIGN your name _____ Date _____

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CHAPTER OVERVIEW

2: Laboratory Methods

2.1: The Scientific Method

2.2: The Metric System and Measurement

2.3: Reading- Descriptive Statistics and Graphing

2.4: Keeping a Lab Notebook

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2.1: The Scientific Method

Adapted from [http://www.biologycorner.com/\(opens in new window\)](http://www.biologycorner.com/(opens in new window))

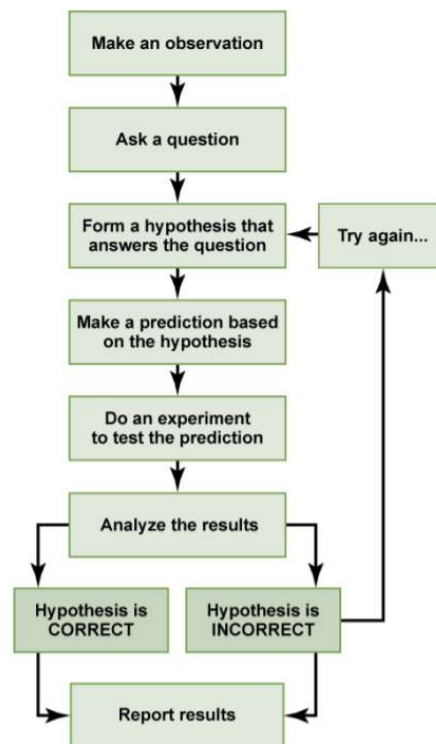
Introduction:

The scientific method is central to the study of biology: it is a process of acquiring and verifying information through experimentation. The general steps of the scientific method are depicted in the figure below. The **hypothesis**, or suggested explanation for the observation, is the basis for setting up experiments. A good experimental design is essential to the scientific method. A few keys to good experimental design include effective use of controls, reproducibility, a large sample size, and multiple trials.

In an experiment, one must determine that any changes that occur are due to investigator manipulation only, there must be some basis for comparison. A **control group** is necessary to establish this basis of comparison. In the control group, everything is kept the same as the experimental group except for the **independent variable**.

The experimental group is being experimented upon. For example, in a drug trial, there will be a group that receives the drug (the experimental group) and a group that receives a placebo (the control group). The drug itself is considered the independent variable and any change(s) that occur because of the drug are considered the **dependent variable**.

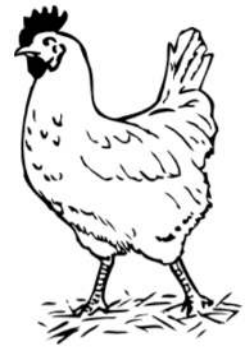
To ensure that it is only the drug causing changes, all other variables must be tightly controlled (such as diet, exercise, smoking, etc.). These are referred to as **controlled variables**.



The Scientific Method. Biology. OpenStax College.

Part 1: The Strange Case of Beriberi:

In 1887, a strange nerve disease attacked the people in the Dutch East Indies. The disease was beriberi. Symptoms of the disease included weakness and loss of appetite; victims often died of heart failure. Scientists thought the disease might be caused by bacteria. They injected chickens with bacteria from the blood of patients with beriberi. The injected chickens became sick. However, so did a group of chickens that were not injected with bacteria. One of the scientists, Dr. Eijkman, noticed something. Before the experiment, all the chickens had eaten whole-grain rice, but during the experiment, the chickens were fed polished rice. Dr. Eijkman researched this interesting case and found that polished rice lacked thiamine, a vitamin necessary for good health.



1. State the problem.
2. What was the hypothesis?
3. How was the hypothesis tested?
4. Do the results indicate that the hypothesis should be rejected?
5. What should be the new hypothesis, and how would you test it?

Part 2: How Penicillin Was Discovered:

In 1928, Sir Alexander Fleming was studying Staphylococcus bacteria growing in culture dishes. He noticed that a mold called Penicillium was also growing in some of the dishes. A clear area existed around the mold because all the bacteria that had grown in this area had died. In the culture dishes without the mold, no clear areas were present. Fleming hypothesized that the mold must be producing a chemical that killed the bacteria. He decided to isolate this substance and test it to see if it would kill bacteria. Fleming transferred the mold to a nutrient broth solution. This solution contained all the materials the mold needed to grow. After the mold grew, he removed it from the nutrient broth. Fleming then added the nutrient broth in which the mold had grown to a culture of bacteria. He observed that the bacteria died, which was later used to develop antibiotics used to treat a variety of diseases.

1. Identify the problem.
2. What was Fleming's hypothesis?
3. How was the hypothesis tested?
4. Do the results indicate that the hypothesis should be rejected?
5. This experiment led to the development of **what** major medical advancement?



Part 3: Identify the Controls and Variables

Smithers thinks that a special juice will increase the productivity of workers. He creates two groups of 50 workers each and assigns each group the same task (in this case, they're supposed to staple a set of papers). Group A is given the special juice to drink while they work. Group B is not given the special juice. After an hour, Smithers counts how many stacks of papers each group has made. Group A made 1,587 stacks; Group B made 2,113 stacks.



Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

What should Smithers' conclusion be?

How could this experiment be improved?



Homer notices that his shower is covered in a strange green slime. His friend Barney tells him that coconut juice will get rid of the green slime. Homer decides to check this out by spraying half of the shower with coconut juice. He sprays the other half of the shower with water. After 3 days of "treatment", there is no change in the appearance of the green slime on either side of the shower.

What was the initial observation?

Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

What should Homer's conclusion be?

Bart believes that mice exposed to radio waves will become extra strong (maybe he's been reading too much Radioactive Man). He decides to perform this experiment by placing 10 mice near a radio for 5 hours. He compared these 10 mice to another 10 mice that had not been exposed. His test consisted of a heavy block of wood that blocked the mice's food. He found that 8 out of 10 of the exposed mice were able to push the block away, while 7 out of 10 of the other mice were able to do the same.



Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

What should Bart's conclusion be?

How could Bart's experiment be improved?



Krusty was told that a certain itching powder was the newest best thing on the market: it even claims to cause 50% longer-lasting itches. Interested in this product, he buys the itching powder and compares it to his usual product. One test subject (A) is sprinkled with the original itching powder, and another test subject (B) was sprinkled with the Experimental itching powder. Subject A reported having an itch for 30 minutes. Subject B reported having an itch for 45 minutes.

Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

Explain whether the data supports the advertisement's claims about its product.

How could this experiment be improved?



Lisa is working on a science project. Her task is to answer the question: "Does Rogooti (which is a commercial hair product) affect the speed of hair growth". Her family is willing to volunteer for the experiment.

Design Lisa's experiment.

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2.2: The Metric System and Measurement

Introduction

The metric system is the world standard for measurement. Not only is it used by scientists throughout the world, but most nations have adopted it as their standard of measurement. All of the measurements done in this course will use the metric system.

The table below shows the standard unit of length, mass, volume, and temperature in the metric system. It also shows the English equivalent.

	Metric	English
Length	meter	39.37 inches
Mass	gram	0.03527 ounces
Volume	liter	1.0567 quarts
Temperature	degree (Celsius)	1.8 degrees Fahrenheit

Meters, grams, and liters (see the table above) form the basis for larger or smaller units. The units are named using these prefixes:

Tera = 1,000,000,000,000

Giga = 1,000,000,000

Mega = 1,000,000

Kilo = 1,000

Hecto = 100

Deca = 10

Deci = 1/10

Centi = 1/100

Milli = 1/1,000

Micro = 1/1,000,000

Nano = 1/1,000,000,000

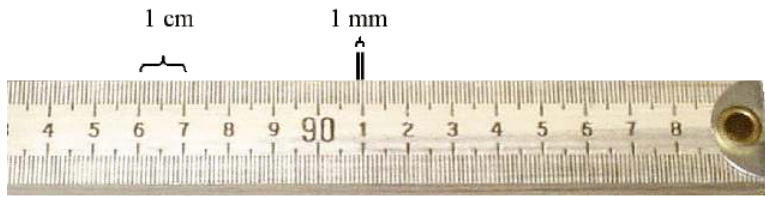
Pico = 1/1,000,000,000,000

The table below shows how meters are related to five other measures of length.

Unit	Length
kilometer (km)	1,000 m (1×10^3 m)
meter (m)	1 m
centimeter (cm)	0.01 m (1×10^{-2} m)
millimeter (mm)	0.001 m (1×10^{-3} m)
micrometer (um)	0.000001 m (1×10^{-6} m)
nanometer (nm)	0.000000001 m (1×10^{-9} m)

Notice that each of the units in the table above are related to meters by a multiple of 10.

The photograph below shows the end of a meter stick. The 90 cm mark can be seen in the center of the photograph. One meter = 100 cm. Notice that each centimeter is divided into 10 mm.



The tables below show similar units based on grams (mass) and liters (volume).

Unit	Mass
metric ton (t)	1,000 kg or 1,000,000 g (1×10^6 g)
Kilogram (kg)	1,000 g (1×10^3 g)
gram (g)	1 gram
milligram (mg)	0.001 g (1×10^{-3} g)
microgram (ug)	0.000001 g (1×10^{-6} g)
nanogram (ng)	0.000000001 g (1×10^{-9} g)

Unit	Volume
kiloliter (kl)	1,000 liters (1×10^3 l)
liter (l)	1 liter
milliliter (ml)	0.001 liter (1×10^{-3} l), 1cm^3
microliter (ul)	0.000001 liter (1×10^{-6} l)

Notice in the table above that one milliliter (ml) equals one cubic centimeter ($1\text{ ml} = 1\text{ cc}$ or cm^3).

Metric Conversions

Exponents

The table below shows how numbers can be written using exponents. For example, a second way to write the number 1,000 is 1×10^3 .

$$10^0 = 1$$

$$100 = 1 \times 10^2$$

$$1000 = 1 \times 10^3$$

$$10,000 = 1 \times 10^4$$

$$0.01 = 1 \times 10^{-2}$$

$$0.001 = 1 \times 10^{-3}$$

Examples

$$256 = 2.56 \times 10^2$$

$$3287 = 3.287 \times 10^3$$

$$0.055 = 5.5 \times 10^{-2}$$

Exponents are useful when writing numbers that are very large or very small. For example the number 1,930,000,000,000,000,000 is easier to write as 1.93×10^{18} .

Decimal Point

Metric conversions are done by moving the decimal point. When converting a large unit such as meters to a smaller unit such as millimeters, the decimal point is moved to the right. When converting smaller units to larger units, the decimal point is moved to the left. You must subtract the exponents in order to determine how many places to move the decimal point.

Larger unit - move decimal point to the left to make a smaller number

	10^{12}	tera (T)
	10^9	giga (G)
	10^6	mega (M)
	10^3	kilo (k)
	10^2	hecto (h)
	10^1	deca (da)
	10^0	
	10^{-1}	deci
	10^{-2}	centi (c)
	10^{-3}	milli (m)
	10^{-6}	micro (μ)
	10^{-9}	nano (n)
	10^{-12}	pico (p)

Smaller unit - move decimal point to the right to make a larger number

Examples

Convert 2.6 cm to μm .

This problem is solved by subtracting the exponents. The exponent for cm is -2; the exponent for μm is -6. Subtract the two numbers: $(-2) - (-6) = 4$. Therefore, to convert 2.6 cm to μm , you must move the decimal point 4 places to the right.

2.6 cm = 26000 μm

Convert 57 μm to cm.

The exponent for μm is -6. The exponent for cm is -2. You must subtract these two numbers to determine how many places to move the decimal point. $-6 - (-2) = -4$. The negative sign indicates that you must move the decimal point 4 places to the left.

57 μm = 0.0057 cm

Rounding

Several of the questions in this exercise ask you to round your answers. Rounding a number to the nearest 0.1 means that your answer should display one digit to the right of the decimal point. For example, the number 0.526 becomes 0.5. Similarly, rounding a number to the nearest 0.01 means that your answer should display two digits to the right of the decimal point. The number 0.526 rounded to the nearest 0.01 becomes 0.53. Notice that the 2 in 0.526 is rounded up to 3 (0.53) because the digit to the right of the 2 is 6. If the number to the right of the last digit being displayed is 5 or greater, the displayed number is increased by 1.

Examples

The number 0.4382251 rounded to the nearest 0.1 is 0.4.

The number 0.4382251 rounded to the nearest 0.01 is 0.44.

The number 0.4382251 rounded to the nearest 0.001 is 0.438.

The number 0.4382251 rounded to the nearest 0.0001 is 0.4382.

Laboratory Exercise

Record your answers to the questions below in your notebook. Do not use scientific notation (exponents) or fractions in your answers to the questions below. Write all of the zeros.

Length

Measurement of Length

Measure the width of a standard 8.5×11 inch page using a small plastic ruler or a meter stick. Record your measurement in 1) millimeters, 2) centimeters, and 3) meters. Record your answers on the answer sheet.

Use a meter stick to measure the width of the laboratory table as shown by the red line in the photograph below. Record your measurement in 4) millimeters, 5) centimeters, and 6) meters.



Click the photograph to view an enlargement.

7) Which unit of measurement (kilometer, meter, centimeter, millimeter, micrometer, or nanometer) would be most appropriate for measuring the width of this room?

Conversions of Length

Perform the following conversions.

8) $1 \text{ m} = \underline{\hspace{1cm}} \text{ cm}$.

9) $1 \text{ cm} = \underline{\hspace{1cm}} \text{ m}$.

10) $3.57 \text{ mm} = \underline{\hspace{1cm}} \text{ um}$.

11) $452 \text{ cm} = \underline{\hspace{1cm}} \text{ mm}$.

12) $0.04 \text{ um} = \underline{\hspace{1cm}} \text{ mm}$

13) $37.6 \text{ nm} = \underline{\hspace{1cm}} \text{ mm}$

14) $52 \text{ nm} = \underline{\hspace{1cm}} \text{ um}$

15) $0.05 \text{ um} = \underline{\hspace{1cm}} \text{ nm}$.

16) $4.3 \text{ m} = \underline{\hspace{1cm}} \text{ um}$

17) $4206 \text{ mm} = \underline{\hspace{1cm}} \text{ cm}$

18) $0.046 \text{ mm} = \underline{\hspace{1cm}} \text{ nm}$

19) $4.8 \text{ cm} = \underline{\hspace{1cm}} \text{ um}$

Use the following information to perform the calculations below.

Metric to English: $1 \text{ meter} = 39.37008 \text{ inches} = 3.28084 \text{ feet}$

English to Metric: $1 \text{ inch} = 0.0254 \text{ meters}$; $1 \text{ foot} = 0.3048 \text{ meters}$

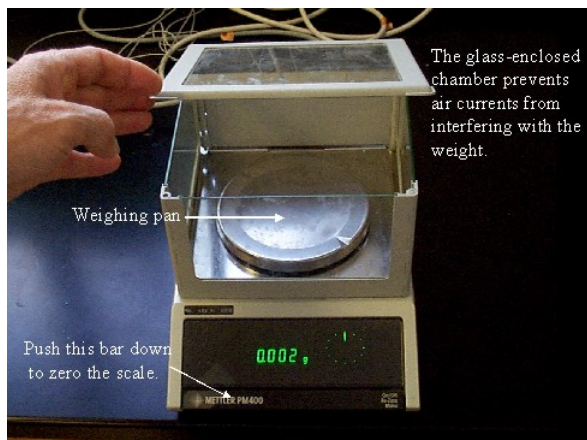
20) $8.53 \text{ inches} = \underline{\hspace{1cm}} \text{ m}$ Round your answer to the nearest 0.001 m.

21) 12 feet, 3 inches = _____ m **Round your answer** to the nearest 0.01 m. [Hint: First, convert 12 ft. 3 inches to feet. It **is not** 12.3 feet.]

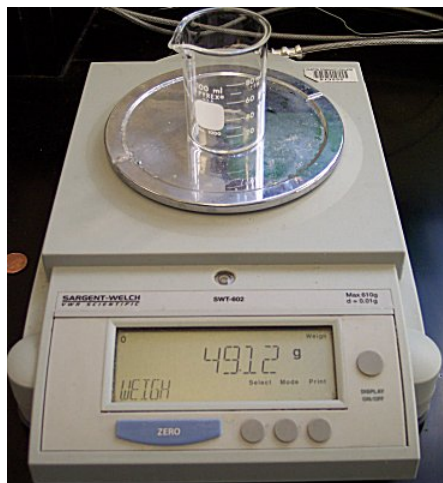
Mass

Measurement of Mass

The laboratory scale shown below uses weight to determine mass. It has a sensitivity of 0.001 g. Due to its sensitivity, moving air will cause it to fluctuate. The glass chamber surrounding the weighing pan prevents air currents from interfering with the measurement.



The scale in the photograph below has a sensitivity of 0.01 g. The scale can be set to zero by pressing the zero (tare) button on the lower left part of the scale.



Place a small beaker on the pan of the scale and zero it by pressing down on the zero (tare) button located on the front of the scale. Place a penny in the beaker to obtain its mass.

22) How much does the penny weigh in grams?

Remove the beaker from the scale and weigh the penny without using the beaker. You must first zero the scale before weighing the penny.

Conversions of Mass

Perform the following conversions.

23) 37 g = _____ mg

24) 0.047 mg = _____ g

25) $45.36 \text{ g} = \underline{\hspace{1cm}} \text{ kg}$

Use the following information to perform the calculations below.

Metric to English: $1 \text{ g} = 0.0352739 \text{ ounces} = 0.0022046 \text{ pounds}$

English to Metric: $1 \text{ ounce} = 28.34951 \text{ grams}$; $1 \text{ pound} = 453.5924 \text{ grams}$

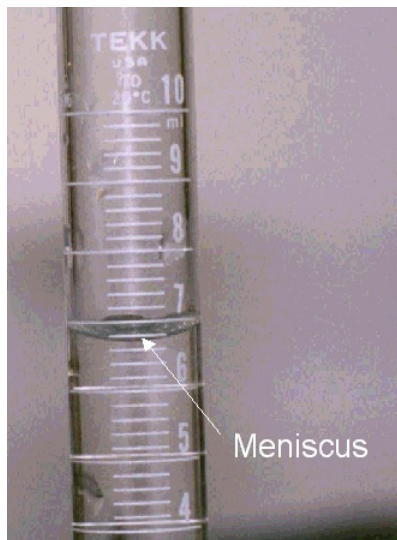
26) $150 \text{ pounds} = \underline{\hspace{1cm}} \text{ kg}$ Round your answer to the nearest 0.01 kg.

27) $3 \text{ oz} = \underline{\hspace{1cm}} \text{ g}$ Round your answer to the nearest 0.01 g.

Volume

Measurement of Volume

28) Obtain a 10 ml graduated cylinder (shown below) and add some water to it. Hold the graduated cylinder in a vertical position at eye level and read the number of milliliters of water that are in the cylinder. Be sure to read the water at the bottom of the meniscus. The arrow points to the bottom of the meniscus in the photograph below. What is the volume of water in your cylinder?



29) Use a 50 or 100 ml graduated cylinder to determine the amount of liquid that a test tube can hold (it's volume).



How did you determine the volume of the test tube?

Conversions of Volume

30) $42 \text{ ml} = \underline{\hspace{1cm}} \text{ liters}$

31) $27 \text{ ul} = \underline{\hspace{1cm}} \text{ liters}$

32) $3.6 \text{ l} = \underline{\hspace{1cm}} \text{ ml}$

33) $1 \text{ ml} = \underline{\hspace{1cm}} \text{ ul}$

Sometimes volume is measured using cubic centimeters (abbreviated cc or cm^3). One cubic centimeter equals one milliliter (1cc = 1ml).

34) 27 liters = _____ cc (or cm^3)

Use the following information to perform the calculations below.

Metric to English: 1 liter = 1.056688 quarts = 0.2641721 gallons

English to Metric: 1 quart = 0.9463529 liters; 1 gallon = 3.785412 liters

35) 2.3 quarts = _____ liters [Round your answer](#) to the nearest 0.01 liter.

36) 0.5 gallons = _____ liters [Round your answer](#) to the nearest 0.01 liter.

Temperature

Measurement of Temperature

The following temperature measurements should be done in Celsius.

37) Measure and record the temperature of the air in the laboratory room.

38) Measure and record the temperature of ice water.

39) Measure and record the temperature of boiling water.

Conversions of Temperature

The temperature in Fahrenheit can be converted to Celsius using the formula:

$$^{\circ}\text{C} = \frac{5}{9} (^{\circ}\text{F} - 32) \quad (2.2.1)$$

For example, to convert 60° F to ° C, subtract 32 (=28), multiply it by 5 (=140) and divide it by 9 (=15.56).

The steps listed above are performed in reverse order to convert Celsius to Fahrenheit. The equation is below:

$$^{\circ}\text{F} = \left(\frac{9}{5} ^{\circ}\text{C} \right) + 32 \quad (2.2.2)$$

For example, 20° C is converted to ° F by multiplying it by 9 (= 180), dividing it by 5 (= 36), and adding 32 (=68).

40) 72° F = _____ °C For this one, use the formula $^{\circ}\text{C} = \frac{5}{9} (^{\circ}\text{F} - 32)$. [Round your answer](#) to the nearest 0.1.

(Note- If you do not have a calculator, use the one on the computer. Click Start, Programs, Accessories, Calculator).

41) 37° C = _____ °F For this one, use the formula $^{\circ}\text{F} = \left(\frac{9}{5} ^{\circ}\text{C} \right) + 32$

Contributors

- [Michael J. Gregory, Ph.D. \(Clinton Community College\)](#)

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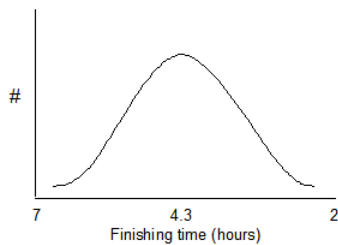
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2.3: Reading- Descriptive Statistics and Graphing

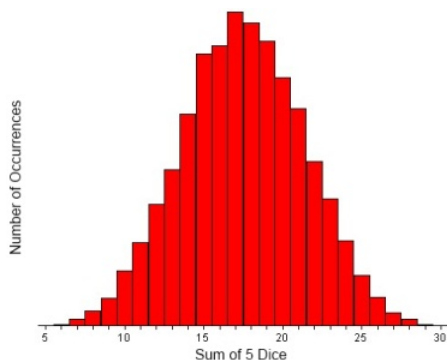
Descriptive Statistics

Normal Distribution

For many kinds of biological data, if the frequency (or number) of data points is plotted on the Y-axis, a bell-shaped curve may be produced as the one shown below. Data sets that produce a bell-shaped curve are said to be normally distributed. For example, suppose that 10,000 runners finish a distance race. A few of the finishers will be among the fastest runners and a few will be the slowest. Most of the finishers will be in the middle. The plot below shows the expected results for finishers of a marathon.



According to the Central Limit Theorem, the sums (or means) of random numbers are normally distributed. An example will illustrate this point. Suppose that we roll 5 dice and add the five numbers from the roll. The smallest number that we could get is 5, and the largest number that we could get is 30. We could then roll the dice again and sum all of the numbers for a second data point. If we repeated this a large number of times, the results would be normally distributed. A Java applet is available at the University of South Carolina website, which will roll the dice a large number of times and create a graph of the result. An example of results for 5 dice rolled 10,000 times is below.



Notice that most of the numbers are in the range of 15 to 20 with very rolls producing 5 or 30. Repeat the procedure with 2 dice and notice that most rolls are 7 with relatively few 2s and 12s.

Many kinds of biological data have a normal distribution as shown above. For example, we could plot the weights or heights of people in a population or their scores on exams. A large sample size will likely show that the data are normally distributed because many biological characteristics are due to more than one gene and the genes often act additively.

Central Tendency

Measures of central tendency are used to describe a "typical" number in a data set. There are different ways to find a typical number and there are advantages and disadvantages to each. Two commonly used measures of central tendency are the mean and the median. These are described below.

Mean

The mean is the average. The mean of a group of numbers is obtained by adding the numbers and then dividing the sum by the total number of data. For example, suppose that a population biologist obtains the following population estimates for waterfowl in six lakes:

Data: 400, 200, 220, 210, 340, 250

The mean of the six numbers below is equal to the sum (1620) divided by the number of data points (6) = 270.

Median

The median is the middle observation. It is obtained by arranging the data in increasing (or decreasing) order. The number that is in the middle is the median.

Data: 27, 40, 3, 51, 34

Data arranged in ascending order: 3, 27, 34, 40, 51

The median for this data set is 34.

If there is an even number of data points, there will be two middle numbers. In this case, the value that is half-way between the two middle numbers is the median. This is equivalent to taking the mean of the two middle numbers.

Data: 400, 200, 220, 210, 340, 250

Data arranged in ascending order: 200, 210, 220, 250, 340, 400

The numbers 220 and 250 are both the middle numbers. The median is therefore the mean of 220 and 250. It is 235.

Which to use- Some Disadvantages of Each:

Mean – The mean gives a good representation of the data if the data are **normally distributed**. However, the mean is influenced by outliers, particularly if the number of data points is small. If there are outliers, the median (discussed below) may be better because it is more immune to this sort of bias. For example, suppose that a population estimates for waterfowl in seven lakes are:

Population estimates for waterfowl

	400
	200
	220
	210
	340
	250
	44,000

The last number in this data set does not seem to belong with the rest; it is an outlier. The typical lake seems to have approximately 200 to 400 birds but the mean for this data set is 6,517. The median (250) may be a better measurement of central tendency.

Median – The median is useful if there are outliers or if the data distribution is not **normal** (a bell-shaped curve) as discussed above. However, the median may not be a representative measure of central tendency if the sample size is small. It is affected by fluctuations in sample data, particularly if the sample is small. For example, suppose that two samples are taken from a population as shown below:

1st sample: 1, 5, 8, 12, 13 Median = 8, Mean = 7.8

2nd sample: 1, 5, 6, 12, 13 Median = 6, Mean = 7.4

Notice that a slight difference in sample data produces a larger change in the median than in the mean.

With a small number of data points, it is possible that the median may not be a central number. In the following data set, the median is 0, which is not a representative number.

52, 50, 48, 0, 0, 0, 0.

Presenting Data

Tables and graphs are convenient for presenting data. They present the data in an organized format, enabling the reader to find information quickly.

Tables

Tables should be numbered sequentially beginning with “Table 1.” Include a descriptive title. The title should enable the reader to understand the table without reading the rest of the document. When creating tables, be sure to state the units of all measurements. Some examples are minutes, milliliters, parts per million, etc.

Example

Table 1. Number of bird species observed in 10 different woodlots in Clinton County, NY on January 18, 2006. Each bird count was done by two observers over a 1-hour period beginning at 8:00 AM.

Number of bird species observed in 10 woodlots

Size of Woodlot (ha)	# Bird Species
3.3	11
8	13
3.6	13
13	14
1.1	7
11.0	14
7.4	12
6.6	14
8	12

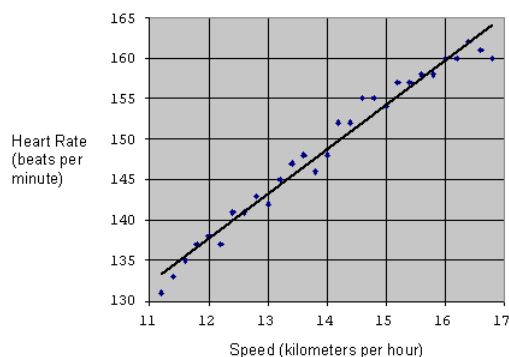
Graphs

Graphs, drawings, and other illustrations are called “Figures.” They should be numbered and titled just like tables.

The scale used on each axis should cover the values in the data set. For example, if you are graphing 52 to 87, start at 45 or 50 and go to 90 or 95.

Variables

The data in the graph below was obtained by measuring the heart rate of a runner while running at different speeds. As can be seen, when the runner ran faster, the heart rate increased.



Heart rate and speed are both referred to as **variables** because their value changes; it is not fixed. It is possible that heart rate depends on speed, so it is called a **dependent variable**. Speed does not depend on heart rate; it is an **independent variable**.

Dependent variables should be plotted on the Y-axis (vertical axis). Independent variables should be plotted on the X-axis (horizontal axis).

Bar Graphs

Bar graphs are best when the data are in groups or categories.

The data in the example below come from population counts of several different kinds of mammals in a woodlot in Clinton County, NY in July 2006.

Gray squirrel – 8

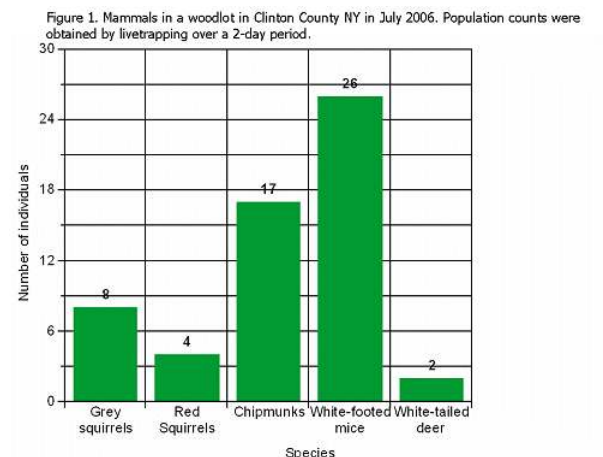
Red squirrels – 4

Chipmunks - 17

White-footed mice – 26

White-tailed deer – 2

A bar graph is best for these data because they are categories; it is not possible to have a data point that is between gray squirrels and red squirrels.



Line graphs

Line graphs are used when the data are continuous.

The data in the example below are pH measurements of a pond in Clinton County, NY on 7/11/09. Samples of water were obtained every 2 hours beginning at 1:00 AM and ending at 11:00 PM.

1:00 AM – 5.3

3:00 AM – 5.2

5:00 AM – 5.1

7:00 AM – 5.2

9:00 AM – 5.3

11:00 AM – 5.5

1:00 PM – 5.8

3:00 PM – 6.0

5:00 PM – 6.1

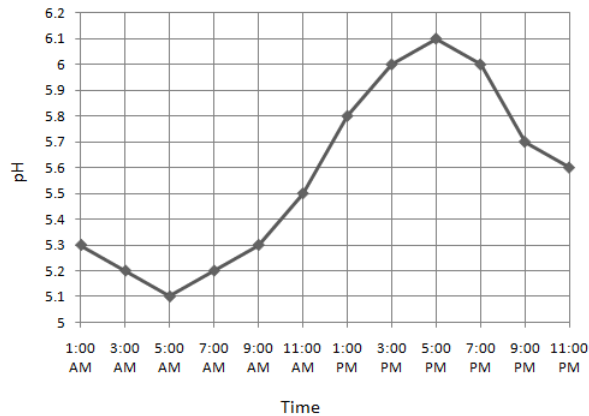
7:00 PM – 6.0

9:00 PM – 5.7

11:00 PM – 5.6

A line graph is appropriate because the data are continuous. Although the researcher in this experiment did not measure the pH at 2:00 AM, it is possible that she could have done that. A line graph shows pH values for time periods that are between the times when samples were taken.

Figure 1. Change in pH of a pond in Clinton County NY in July 2009. Measurements were obtained from samples of water taken 2 m from the shore at a depth of 10 cm.



Scatter Plots

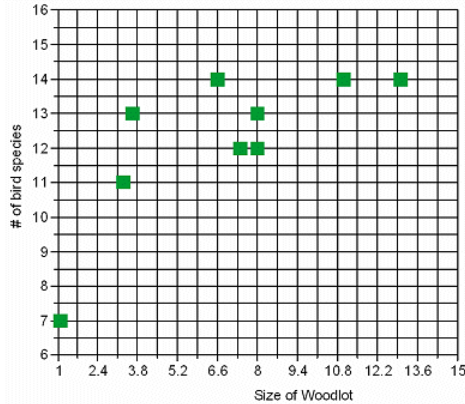
Scatter plots are useful to see if there is a relationship between two variables. It is not appropriate to connect the data points with a line because each data point is independent of the points next to it; the measurements were not taken in a sequential order. If the data points were connected with a line, an increase or decrease from one point to the next would not provide any useful information.

Suppose that a researcher wants to learn if there is a relationship between the size of the forest and the number of bird species that live in the forest. She collects the following data from different woodlots around Clinton County, NY in January 2006.

Data

Size of Woodlot (ha)	# Bird Species
3.3	11
8	13
3.6	13
13	14
1.1	7
11.0	14
7.4	12
6.6	14
8	12

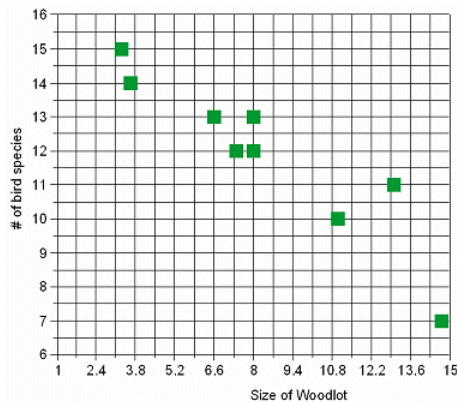
Figure 3. The number of bird species observed in 10 different woodlots in Clinton County, NY on January 18, 2006. Each bird count was done by two observers over a 1-hour period beginning at 8:00 AM.



The points on the graph indicate that larger woodlots generally have more bird species.

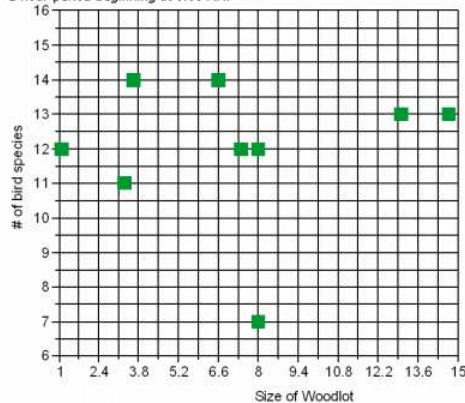
Suppose that the data collected produced the graph below instead of the one shown above. The graph below shows a negative relationship; larger woodlots have fewer bird species.

Figure 3. The number of bird species observed in 10 different woodlots in Clinton County, NY on January 18, 2006. Each bird count was done by two observers over a 1-hour period beginning at 8:00 AM.



Suppose that the data collected produced the graph below, instead of the ones shown in the previous two examples. The graph below shows that size of the woodlot does not seem to be related to the number of bird species.

Figure 3. The number of bird species observed in 10 different woodlots in Clinton County, NY on January 18, 2006. Each bird count was done by two observers over a 1-hour period beginning at 8:00 AM.



Rounding and Significant Digits

It is often desirable to round numbers. For most purposes in this laboratory course, numbers should be rounded to 3 significant digits. Some examples below illustrate this concept.

The number 35,832,487 can be rounded to 35,800,000. We use the three digits that are furthest to the left; the rest become zeros. The number 35,852,487 becomes 35,900,000. If the number to the right of the 3rd digit is 5 or greater, the 3rd digit is rounded up. If it is less than 5, it is rounded down.

The number 2.4815 becomes 2.48. The number 2.4855 becomes 2.49.

Example

The table below shows the number 12753122 rounded to several different significant digits.

Number of significant digits	Number
1	10000000
2	13000000
3	12800000
4	12750000

The table below shows the number 0.4382251 rounded to several different significant digits

Number of significant digits	Rounded Number
1	0.4
2	0.44
3	0.438
4	0.4382

Contributors

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2.4: Keeping a Lab Notebook

Learning Objectives

- Understand the importance of a well-kept notebook.
- Learn how to enter information and maintain a legal scientific notebook.

Upon completion of this lab, students will be able to:

- Record information into a notebook in a legally acceptable format.
- Format a table of contents.
- Make appropriate corrections to their notebook.
- Engage in good laboratory practices.

Introduction

Regardless if you work at a biotechnology company or in an academic research lab, keeping a lab notebook is a requirement. The Food and Drug Administration's (FDA) handbook states, "if it isn't written down, it wasn't done." The lab notebook is considered a legal document and can be used in court to settle patent disputes or to report a specialist's findings in paternity suits or criminal cases. Oftentimes, the lab notebook is used as a starting point for other scientists who work in the lab. Lab notebooks are always maintained for the following reasons:

- To record the steps an individual has carried out and to document their observations
- To establish ownership in case of a patent dispute or other legal issues
- To establish guidelines used to evaluate the process in which a product was made and to evaluate the product itself
- To follow the production of a product through the manufacturing process
- To create a contract between a company and consumers and/or between a company and regulatory agencies
- To demonstrate the procedure was done correctly
- To develop, follow, and evaluate standard operating procedures (SOP)

Without documentation, even quality work is worthless. In the event of an experiment failing, the documentation allows for a scientist to review their protocol and make adjustments for future experiments. As stated above, in industry, lab notebooks are legal documents. They are used to determine product quality, patent rights, and liability. Notebooks are always treated as if they will be used in court because the consequences of not doing so could be devastating to a company or research program.

Lab notebooks are kept to document and organize your experimental plans and data. Every lab requires each researcher to keep one. Yet no two scientists organize their lab notebooks identically, and there isn't one "right" way for you to keep yours. There are some common elements that all lab notebooks share and some important habits you should develop in keeping your notebook for this class. All lab notebooks should be...

1. Complete

Your notebook is a place to collect descriptions of experimental goals, experimental procedures, all the data you collect, and your interpretations of results. Numerical data and calculations should be written directly into your notebook, not on scraps of paper to be entered later. Data in the form of a photo should be taped into your notebook. Printouts and X-ray films can also be taped into your notebook or if reams of paper and large films are being collected, they can be organized in a separate binder and referenced in your notebook.

2. Organized

Some scientists arrange their notebooks by date, others by the question being tested. What works best depends on the research itself and the researcher. Since this class has four experimental modules that are performed sequentially, your notebook will, by default, be organized by both date and project. You will keep a record of every lab meeting, including both the date and the module/day in your notebook.

3. Up to Date

For this class, that means coming to lab with the date, module/day, title, purpose, and description already entered in your notebook. It will occasionally be helpful to have data tables ready or some calculations performed as well. "Up to date" also means leaving lab with your protocol and any amendments you made to it, data, and perhaps some interpretation entered in your notebooks. Your notebook does not need a table of contents, but you should realize that most research notebooks do.

4. Permanent

Use pen when you write in your notebooks.

Some Other Things You Should Know About Lab Notebooks

- They are the property of the research lab itself. Researchers who join the lab after you have left it will get to know you through the notebooks you have kept there. Ideally, your notebooks will reflect your most organized, clear, and thoughtful side.
- They are legal documents. Labs in the industry have special rules about lab notebooks since patent disputes and court cases often hinge on lab notebook entries.
- They are both personal and public. It is considered impolite and an invasion of privacy to read someone else's notebook without their permission. Most people are happy to show you their notebooks when asked.

Evaluation: Grading your Notebook

Your notebook pages will be evaluated by looking for these components

LAB NOTEBOOK
Date of experiment
Module/Day #
Title for experiment
Brief statement of purpose
Protocol (note the Things to Remember below)
Tables for data entry
Calculations entered
Data labeled
Summary/Interpretation
Initials of all group members

Things to Remember

Remember the goal of your notebook is to help you repeat your experiments with the same results. Information you should record includes:

- Centrifuge settings: temperature, speed, time
- Incubator settings: temperature, time, and shaking speed if applicable
- Size and types of tubes used
- Buffers (and their pH)
- Media
- Dilutions and how they were prepared
- Concentrations
- Volumes used
- Washes: number, volumes, temperature, solutions used
- Antibody: dilutions, lot or tube #s
- Electrophoresis: agarose or acrylamide percentages, voltages, times

- The names of people who helped you with your experiment

You should also note any changes to the protocol such as:

- unexpected delays ("waterbath wasn't ready so tubes kept on ice for one hour")
- unanticipated conditions ("roller drum found off in AM")
- unusual observations ("a large number of cells seemed to be floating").

Put it into Practice: Setting Up Your Scientific Notebook

1. Obtain a **bound notebook**, such as a composition book. Spiral notebooks are not acceptable.
2. Use only blue or black pen to make entries. **NO PENCIL ALLOWED.**
3. Label the outside of your notebook with the information:
 - Names
 - Course and Section
 - Semester
4. Make pages 1-4 the Table of Contents. Write "Table of Contents" at the top of each of these pages. Record the title of each lab or entry and the page number for each.
5. Each page in the notebook must be numbered. Number each page of the notebook in the upper corner with the first page of the notebook being "page 1." The back of the first page will be "page 2" and so on.
6. Record the date of each entry at the top of each page.
7. All group members present should initial the bottom of each page.
8. For each experiment (or activity) that you perform, you should include the:
 - purpose for the experiment
 - the materials and methods you use
 - any data tables
 - figures or graphs properly labeled with a title and a conclusion.
9. Do not erase errors. Just draw a single line through an erroneous entry, then add your initials. Enter the correct entry nearby.
10. Never leave blank spaces. Draw a diagonal line through all open or blank space.
11. If an entry continues on a different page, include "go to page ____" on the bottom right hand of the page to let the reader know where the rest of the information is located.
12. Graphs and other small sheets of paper can be pasted into the notebook using tape.
13. Always include enough details for someone else to successfully duplicate the work you have recorded.
14. Label all figures, tables and calculations. Figures should be labeled on the **bottom left** and tables are labeled on the **top left**.
15. Never remove pages from your notebook.

Practice Activity

Your instructor will provide information and data to enter into your notebook.

1. Work with your group to record an appropriate entry and data analysis.
2. Review the guidelines above and determine whether your entry meets the criteria.

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CHAPTER OVERVIEW

3: Introduction to Microscopy and Diversity of Cell Types

- [3.1: Introduction](#)
- [3.2: Procedures](#)
- [3.3: Results](#)
- [3.4: Review Questions](#)
- [3.5: Scientific Nomenclature](#)

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3.1: Introduction

Learning Objectives

- Review the principles of light microscopy and identify the major parts of the microscope.
- Learn how to use the microscope to view slides of several different cell types, including the use of the oil immersion lens to view bacterial cells.
- Learn about the shapes and arrangements of some common types of bacteria.
- Review the taxonomic classification system used in scientific nomenclature.

The first microscope was developed in 1590 by Dutch lens grinders Hans and Zacharias Jansen. In 1667, Robert Hooke described the microscopic appearance of cork and used the term cell to describe the compartments he observed. Anton van Leeuwenhoek was the first person to observe living cells under the microscope in 1675—he described many types of cells, including bacteria. Since then more sophisticated and powerful scopes have been developed that allow for higher magnification and clearer images.

Microscopy is used by scientists and health care professionals for many purposes, including diagnosis of infectious diseases, identification of **microorganisms** (microscopic organisms) in environmental samples (including food and water), and determination of the effect of pathogenic (disease-causing) microbes on human cells. This exercise will familiarize you with the microscopes we will be using to look at various types of microorganisms throughout the semester.

The Light Microscope

What does it mean to be microscopic? Objects are said to be microscopic when they are too small to be seen with the unaided eye—they need to be magnified (enlarged) for the human eye to be able to see them. This includes human cells and many other types of cells that you will be studying in this class. The microscope you will be using uses visible light and two sets of lenses to produce a magnified image. The total magnification will depend on which objective lens you are using—the highest magnification possible on these microscopes is 1000X—meaning that objects appear 1000X larger than they actually are.

resolution vs. magnification

Magnification refers to the process of making an object appear larger than it is; whereas **resolution** is the ability to see objects clearly enough to tell two distinct objects apart. Although it is possible to magnify above 1000X, a higher magnification would result in a blurry image. (Think about magnifying a digital photograph beyond the point where you can see the image clearly). This is due to the limitations of visible light (details that are smaller than the wavelength of light used cannot be resolved).

The limit of resolution of the human eye is about 0.1 mm, or 100 microns (see Table 1 for metric review). Objects that are smaller than this cannot be seen clearly without magnification. Since most cells are much smaller than 100 microns, we need to use microscopes to see them.

The limit of resolution of the light microscope you will be using today is about 0.1 μm , or 100 nm. This means that we can view objects that are 1000X smaller than what we can see with our eyes alone. Biologists typically use microscopes to view all types of cells, including plant cells, animal cells, protozoa, algae, fungi, and bacteria. The nucleus and chloroplasts of eukaryotic cells can also be seen—however smaller organelles and viruses are beyond the limit of resolution of the light microscope (see Figure 1).

Table 1: Metric units commonly used in Microbiology

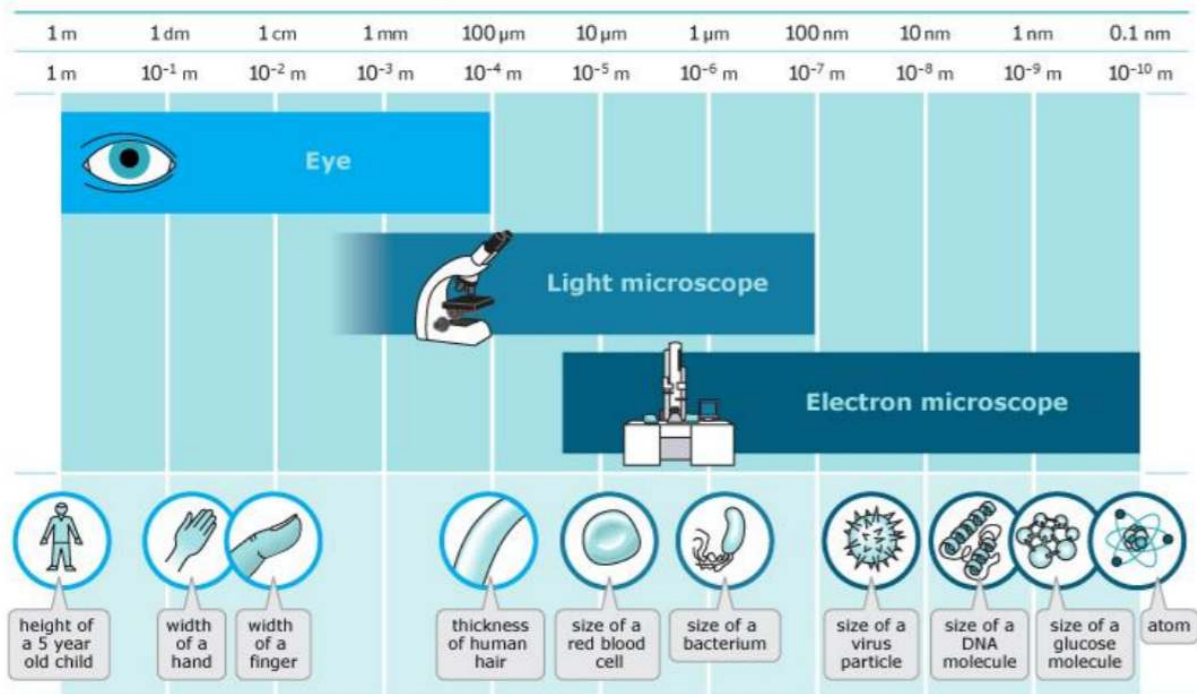
The basic unit of measurement of length in the metric system is the meter.

There are 1000 millimeters (mm) in one meter. $1 \text{ mm} = 10^{-3} \text{ meter}$.

There are 1000 micrometers (microns, or μm) in one millimeter. $1 \mu\text{m} = 10^{-6} \text{ meter}$.

There are 1000 nanometers in one micrometer. $1 \text{ nm} = 10^{-9} \text{ meter}$.

Resolving power of microscopes



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Figure 1.1: Resolving Power of Microscopes

Key Terms

microorganism, magnification, resolution, working distance, parfocal, parcentric, prokaryotic, eukaryotic, bacillus, coccus, spirillum, spirochete, morphology, bacterial arrangements, depth of field, field of view, taxonomic classification

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3.2: Procedures

Use and Care of the Microscope:

When instructed to do so, obtain the microscope assigned to you. Familiarize yourself with the major parts and their functions—you may use the information found at the end of this chapter to guide you.

Basic Guidelines for Using the Microscope

1. Always carry the microscope with **two hands**.
2. Always use the microscope that is assigned to you.
3. Clean the lenses with lens cleaner (Windex) and lens tissue before and after use.
4. Report any problems with the microscope to your instructor immediately.
5. Oil **must** be cleaned off completely before returning the microscope to the cabinet. If you accidentally get oil on the 40X objective, clean it immediately. Microscopes must always be returned to the cabinet clean.
6. Microscopes should always be put away with a low power objective (4X or 10X) over the stage.
7. Always **lift** the microscope to reposition it—do not drag it across the surface of the table!

Total Magnification: The microscope you are using has two sets of lenses that both contribute to the total magnification of the image. The ocular lenses magnify your image 10X. There are 4 different objective lenses—each with a different magnification. The total magnification is calculated as follows:

$$\text{ocular magnification} \times \text{objective magnification} \quad (3.2.1)$$

Since the ocular magnification of our microscope is 10X, determining the total magnification of an object with this microscope simply requires multiplying the objective magnification by 10. (Note: other microscopes may have ocular lenses with a different magnification, for example, 12X.)

? Exercise 3.2.1

Fill in the table below:

Name of Lens	Objective Magnification	Total Magnification
Scanning		
Low		
High-Dry		
Oil Immersion		

Diversity of Cells: There are many types of cells found among the diverse forms of life on the planet. All cells have certain features in common, such as a plasma membrane surrounding the cell, cytoplasm within the plasma membrane, and DNA as the molecule that stores genetic material. However, there is a great deal of diversity among the cells that make up living organisms. Some living organisms are composed of one cell (unicellular); others are composed of many cells (multicellular).

Cells that have a nucleus and other organelles are **eukaryotic**; bacterial cells do not have these intracellular structures—they are **prokaryotic**.

Some types of cells (plants, algae, and some bacteria) are photosynthetic (capable of using light energy to make organic compounds from inorganic materials). Others take in organic molecules as a source of energy.

There are several other similarities and differences between cell types that you will learn about throughout the semester. In today's lab, you will use the microscope to look at various types of cells that are often studied in a Microbiology course.

Examination of different cell types under the microscope

In this activity, you will look at some prepared slides, as well as make a few of your own.

Materials:

- Prepared slides: Blue-green algae, mixed protozoa, bacterial types
- Slides and cover slips
- Pond water sample (if available)
- Live *Saccharomyces* culture
- Methylene blue stain
- Prepared slides (demos): may include Pinworm (*Enterobius*), pinworm eggs, mosquito, yeast cells (*Saccharomyces*), bacterial slides showing varying morphologies and arrangements

Prepared slides: Eukaryotes

Instructions: Use the spaces provided at the end of this exercise to draw pictures as you are viewing the slides.

1. Place your first slide on the mechanical stage and make sure that it is level and held firmly in place. Your sample /smear should be facing upwards.
2. Use the knobs located below the stage to move the slide left and right, and up and down until the stained area of the slide is centered over the light source.
3. Position the scanning power objective (4X) in place over the slide. (Note: objective will “click” into place).
4. Use the coarse adjustment knob to bring the stage up as far as it will go.
 - When using the scanning or low power objectives the **working distance** (the distance between the lens and the slide) is large enough so that the slide will never make contact with the lens. This is not the case when using the high-dry and oil immersion lenses, where the working distance is significantly less. This is why the coarse adjustment knob can only be used with the two low-power lenses.
5. Rotate the coarse adjustment knob away from you until the image comes into focus.
 - You will not be able to make out much detail at this power—the purpose is to find where your specimen is on the slide so that it is easier to locate when you switch to high power. Low power objectives have a large **field of view** (the circular area seen when looking through the microscope) and a large **depth of field** (the thickness of a specimen that is in sharp focus). As magnification increases, both the field of view and depth of field decrease, which is why it is easier to locate your specimen using a low power objective.
6. If needed, use the fine adjustment knob to improve the clarity of the image.
7. After viewing the slide at scanning power, move the slide so that the area you want to focus on is in the center of the field of view.
 - Since your microscope is **parcentric**, when you increase magnification, you will be zooming in at the center of the field of view. Objects that are not centered at low power may be out of the field of view at high power.
8. Rotate the objective lens nosepiece so that the 10X objective is in place over the slide. Re-focus and adjust the light (if needed) under the 10X objective.
 - The microscope you are using is **parfocal**—this means that when it is in focus with one lens in place the same stage position will be in focus with all other lenses. Therefore, when switching objectives, DO NOT change the position of the stage—just click the objective you wish to use into place.
 - Note the differences in the appearance of the cells from when you were using the 4X objective.
9. After focusing at 10X, rotate the objective lens nosepiece so that the 40X objective is positioned over the slide. Re-focus using the fine adjustment knob and adjust the light if needed.
 - Note: Remember that you CANNOT use the coarse adjustment knob at high power (40X or 100X objectives). When you are using the high-power lenses, the lens is very close to the slide (small working distance) therefore using the coarse adjustment knob at high power could result in damage to the lens, damage to the slide, or both.
 - Remember to sketch what you are looking at in the spaces provided.

10. When you are finished looking at the slide, return it to the slide box and proceed to a different slide. Follow the instructions above to view the slide with the 4X, 10X, and 40X objectives in place. Draw diagrams of each.
 - When viewing the mixed protozoa slide, you should scan different areas of the slide, as there are many types of protozoans and algae present on this slide. Some organisms you might see include Volvox, Amoeba, and Paramecium. Note that in most of these cells, the nucleus is clearly visible.
 - Sketch a few areas of this slide with the 10X or 40X objective in place. Use the charts in the lab to identify some of the organisms you see.
11. When you have finished looking at the prepared slides proceed to the wet mounts.

Preparation of wet mounts

These slides will be prepared with live microbes on them. Since the liquid is left on the slide, cover slips are needed to prevent this liquid from touching the lenses.

- Live protozoa: use a dropper to place 1-2 drops of a pond water sample onto a clean microscope slide. Take a cover slip and place it down over the water as shown in the diagram below (try to avoid air bubbles).
- Preparation of a live yeast culture: place one drop of the yeast (*Saccharomyces*) suspension and one drop of methylene blue onto a clean microscope slide, and prepare a wet mount as shown.

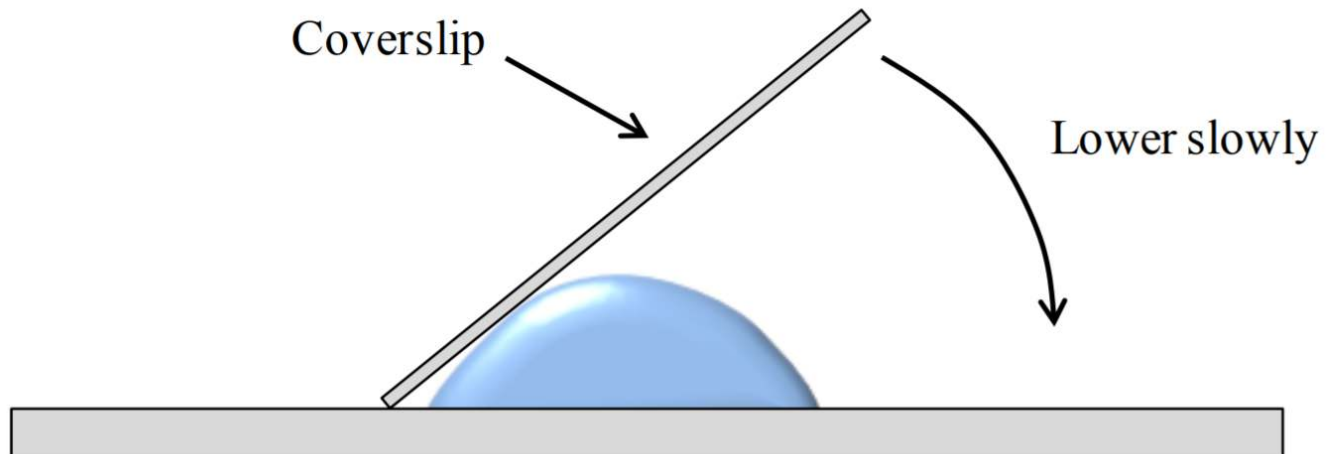


Figure 3.2.1: Preparation of a Wet Mount

Observe these slides under the microscope (use the magnifications suggested by your instructor). As time permits, your instructor may have you examine other types of cells that are set up on demonstration scopes.

Observing bacterial cells under the microscope

In this exercise, you will learn how to use the oil immersion lens and observe some common morphologies and arrangements of bacteria.

1. Obtain a **bacterial types** slide from the slide box and place it on your microscope. Since bacterial cells are very small, you will need to use the highest magnification (100X objective, or 1000X total magnification) to see them clearly—however, as with all slides, you should use a low-power objective lens to focus on the slide before moving to high power.
 - The bacterial types slide may be viewed with all 4 objectives—alternatively, your instructor may ask you to skip some (for example, just use the 10X and 100X objectives). Follow the instructions you receive in class.
 - There are three areas of bacteria on the slide. The area closest to the slide label is the darkest and therefore the easiest to find: the area on the rightmost side of the slide is very faint and you may not be able to detect any stain with your eyes.
2. Focus with a low-power objective on the first area of the slide.

- The cells will still be very small at this magnification, and you will not see any detail.
- 3. Once in focus, switch the objectives to 10X and then to 40X (this step may be skipped). Adjust light and focus as needed.
- 4. You are now ready to use the oil immersion lens. This lens requires the use of immersion oil, which has the same index of refraction as glass, to prevent light from scattering and focus it on your specimen (we need a lot of light to see clearly at this high magnification).
 - Immersion oil **MUST** be used with the 100X lens.
 - Immersion oil should **NEVER** be used with the high dry lens.
- 5. Rotate the nosepiece so that there is no objective over the stage. Add 1-2 drops of immersion oil to the slide right above where your sample is.
- 6. Click the 100X objective into place. If done correctly, you should only need to fine focus a little bit to bring the cells into view.
 - Remember to **NEVER** use the coarse adjustment knob when focusing under 40X or 100X objectives.
 - If you “get lost,” it’s better (and faster) to go back to low power and refocus, then switch back to 100X.
 - Once there is oil on the slide you **CANNOT** use the 40X lens—rotate the nosepiece the other way to use 4X or 10X to refocus.
- 7. Use the spaces provided to draw the cells. At this point, your instructor will discuss the morphology (shape) and arrangements of the cells you are looking at. (See Figure 3.4.2. at the end of the exercise for more information.)
- 8. When you are finished, move the slide to the right to find the second area of cells (middle of the slide), and then to the third area of cells (right side of the slide).
 - The third area of cells is very faint, and the cells are more spread out. If you “get lost”—try going back to find the second area and then carefully move the slide across to the third area while keeping the slide in focus.
- 9. When you are finished, clean all oil off the slide and return it to the slide box.

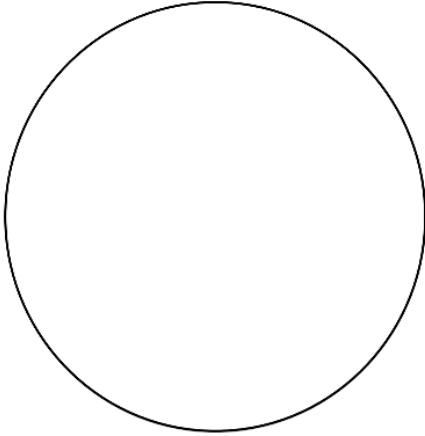
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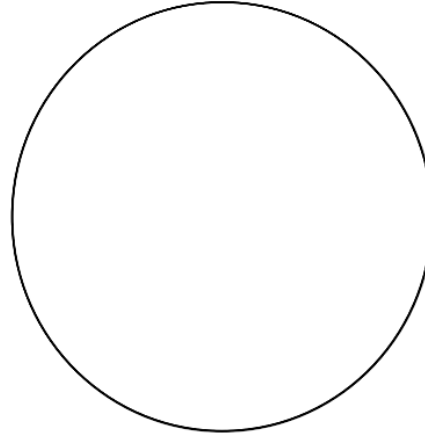
3.3: Results

Use the circles below to draw sketches of the organisms you observe. Remember your Magnification is the **total magnification** (Objective magnification x Ocular magnification). For each sketch, include a brief description of what you observed!

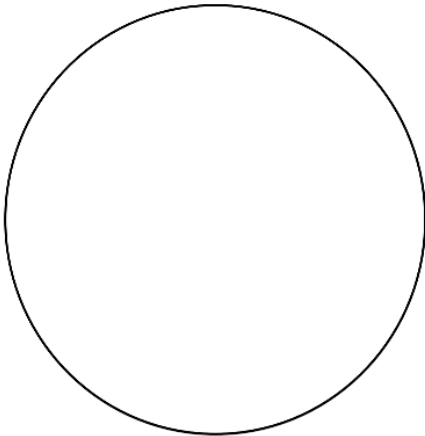
Name of organism _____
Magnification _____



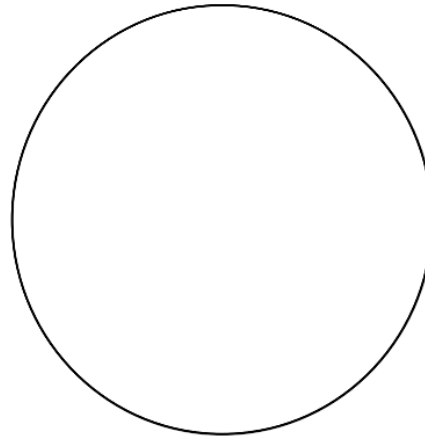
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Magnification _____



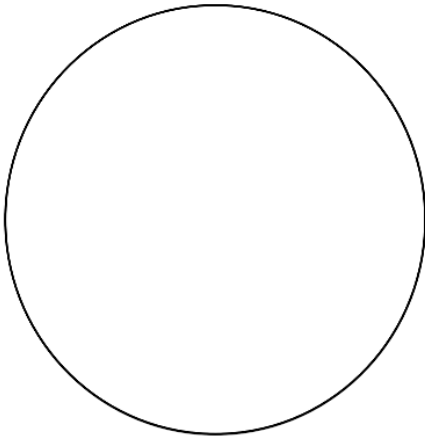
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Magnification _____



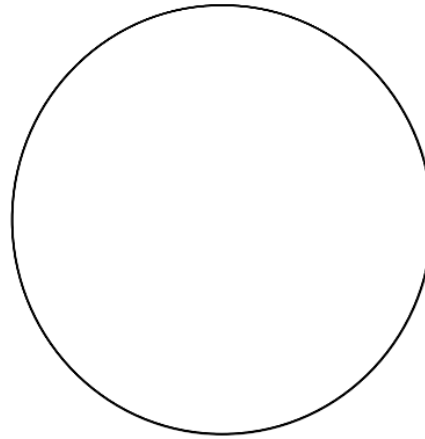
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Magnification _____



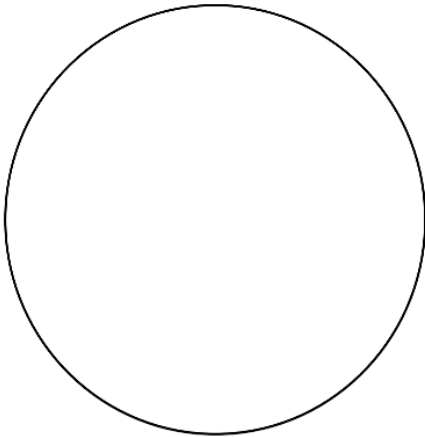
Name of organism _____
Magnification _____



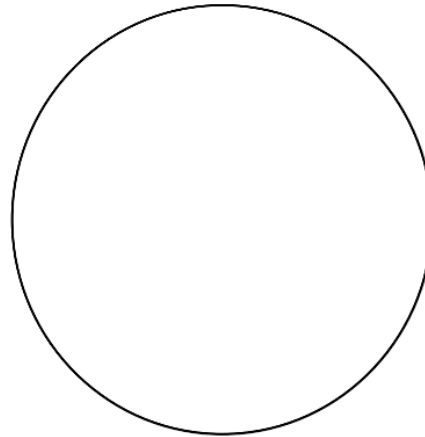
Name of organism _____
Magnification _____



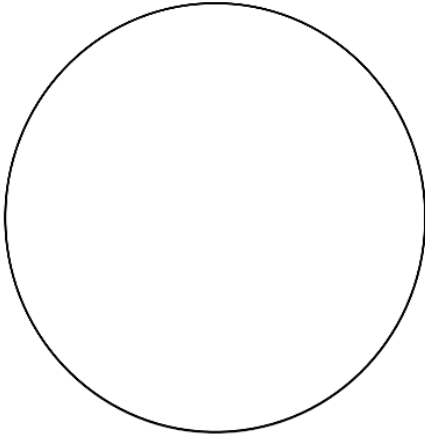
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Magnification _____



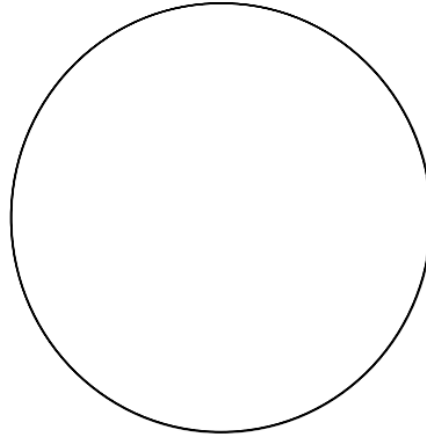
Name of organism _____
Magnification _____



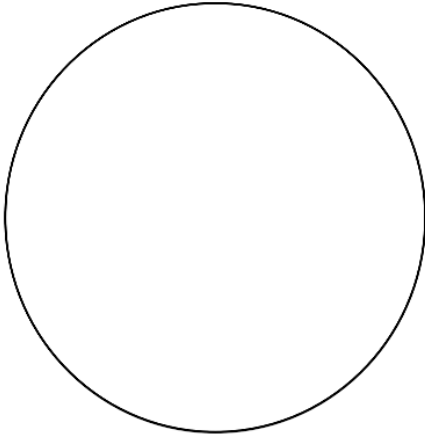
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Magnification _____



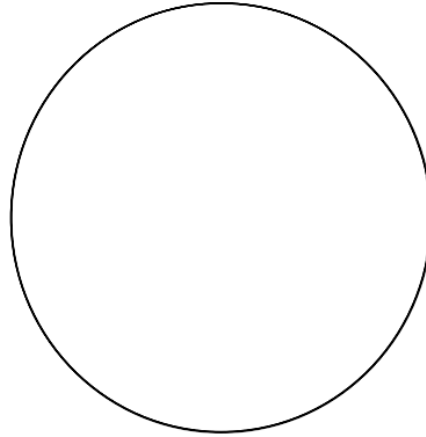
Name of organism _____
Magnification _____



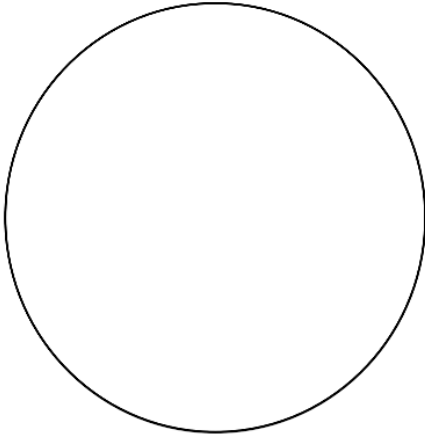
Name of organism _____
Magnification _____



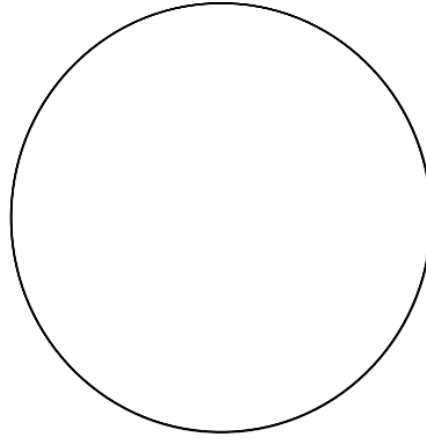
Name of organism _____
Magnification _____



Name of organism _____
Magnification _____



Name of organism _____
Magnification _____



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3.4: Review Questions

Review Questions

1. Why is it important that health care professionals know about microorganisms?
2. What is the main difference between prokaryotic and eukaryotic cells?
3. Why is it important to use immersion oil when using the 100X objective?
4. Are there things that are too small to be seen with a light microscope? Explain.
5. Of the organisms you have looked at today, which are unicellular and which are multicellular?

Unicellular:

Multicellular:

6. Fill in the blanks: As magnification increases, the area of the field of view _____, the depth of the field of view _____, the working distance _____, and the amount of light required _____.
7. Distinguish between morphology and arrangement.
8. What do you think would happen if you tried to view a slide using the oil immersion lens but forgot to add the oil?
9. Use the information below to label the parts of the microscope on Figure 3.3.1.

Parts of the Microscope

- a) **Ocular (eyepiece):** what you look through to view your slide. Our microscopes are binocular, which means they have two eyepiece tubes and both eyes are used (monocular microscopes have only one tube). Typically, ocular lenses magnify an image 10X, although some have other magnifications (ex: 12X). Binocular microscopes allow for adjustment of the distance between pupils so that both eyes can be used to observe one image.
- b) **Objective lenses** are the lenses closest to the stage. Usually, microscopes will have 3-5 lenses of different magnifications (ours have 4—4X, 10X, 40X, and 100X magnifications). Objective lenses are located on a rotating turret to allow for changes in magnification.
- c) **Coarse adjustment knob:** the larger (and outermost) of the two focusing knobs—moves the stage toward or away from the objectives to bring the image into focus at low power.
- d) **Fine adjustment knob:** smaller knob within the coarse adjustment knob—used for “fine-tuning” an image. Only fine focus can be used when the 40X and 100X objectives are in place.
- e) **Stage:** the platform where the slide to be viewed is placed. A mechanical stage holds the slide in place and allows for the movement of the slide to view different areas.
- f) **Illuminator (light source):** found at the base (bottom) of the microscope below the stage.
- g) **Condenser/iris diaphragm assembly:** found directly beneath the stage. This assembly can be raised and lowered using a knob at the side of the microscope. For our purposes, the assembly should be positioned very close to the bottom of the stage. The condenser is a lens that focuses the light from the illuminator onto the specimen. The iris diaphragm controls the amount of light that passes through the specimen. The iris diaphragm can be opened and closed by twisting the ridged ring of the assembly.
- h) **Base:** the bottommost part of the microscope that contains the illuminator.
- i) **Arm:** positions the objective lenses and the oculars above the stage. When moving the microscope, the base should be supported with one hand, while the arm is grasped with the other hand.

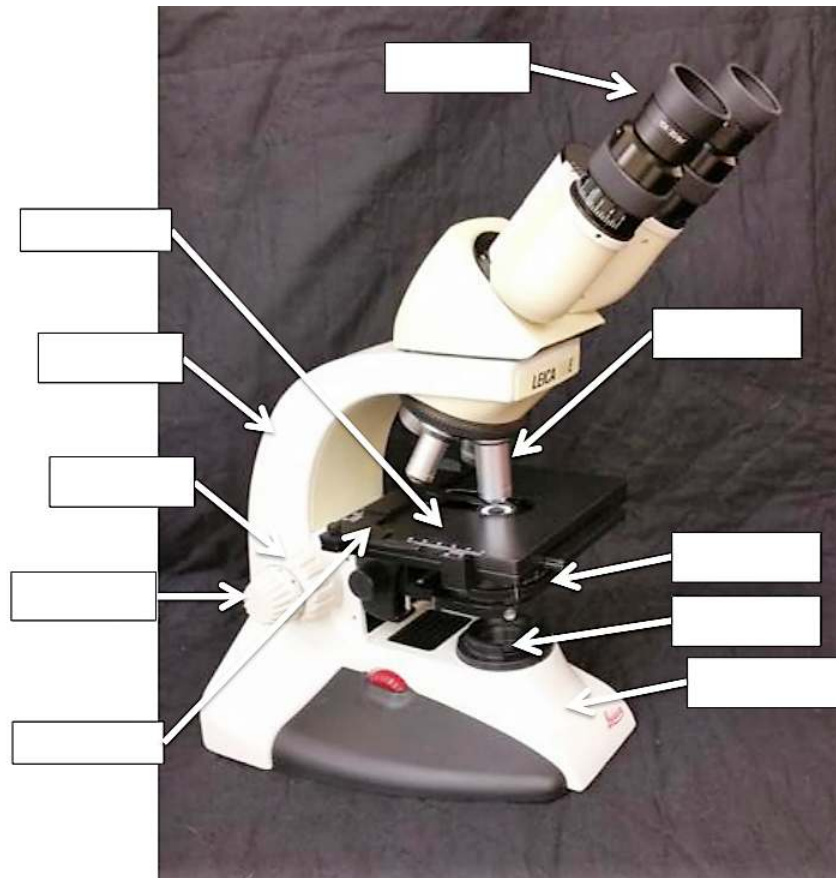


Figure 3.3.1: *Label the microscope diagram*

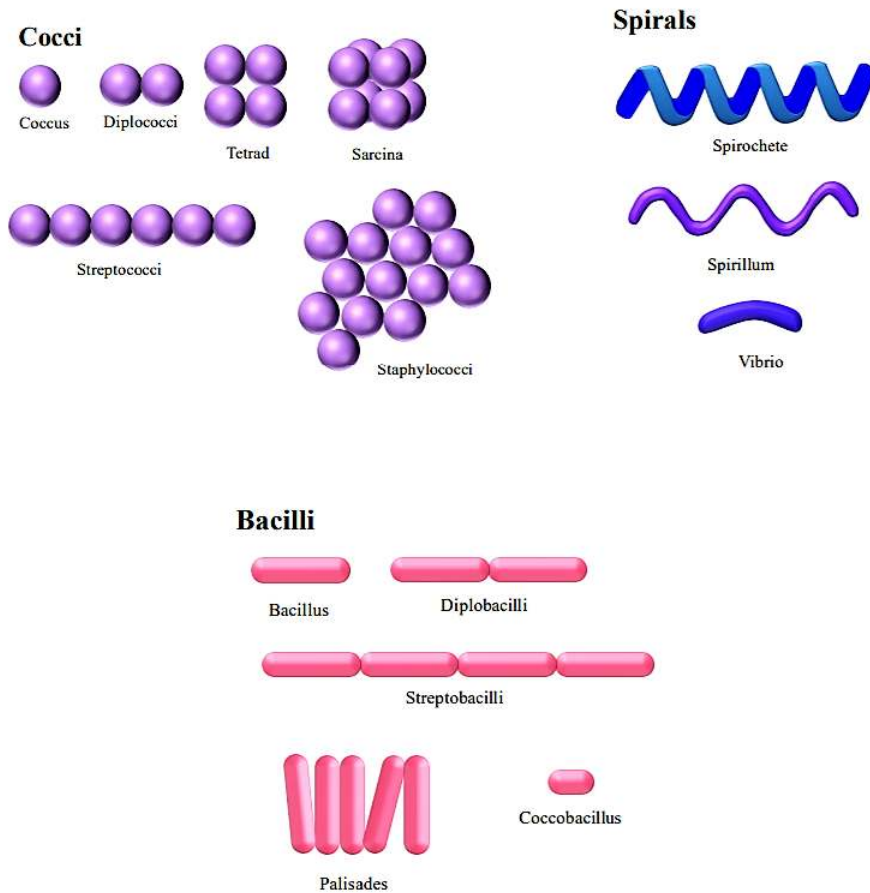


Figure 3.4.2: Common shapes and arrangements of bacteria

Note

There are other less common bacterial morphologies (e.g., filamentous, squares, etc.) that are not shown here.

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3.5: Scientific Nomenclature

Scientific nomenclature is based on a taxonomic classification system. **Taxonomic classification** is a hierarchical system used to classify and compare organisms. There are 8 ranks in this system, listed below in the order of the most general (broadest) to the most specific:

1. Domain
2. Kingdom
3. Phylum
4. Class
5. Order
6. Family
7. Genus
8. Species

Prokaryotic organisms like bacteria may have classifications below the species level, including strain, subspecies, serotype, morphotype or variety. Taxonomic classification indicates how closely organisms are related. For example, two organisms sharing the same Class are more closely related than two organisms sharing the same Phylum.

Binomial nomenclature: The scientific name of an organism consists of two words: the genus name and the specific epithet. The genus name comes first and is always capitalized; once identified it can be abbreviated to a single letter. The second word is known as the specific epithet and is not capitalized. The two words together make up the scientific name or species name. The genus can be used alone (you can refer to the genus *Staphylococcus* or the genus *Bacillus*) but the specific epithet without the genus name has no scientific significance. Scientific names in print should always be either italicized or underlined and should always be underlined when written. For example, the scientific name for human beings is *Homo sapiens* or *H. sapiens*. The scientific name of a bacterium is *Staphylococcus aureus* or *S. aureus*.

The scientific name often includes a description of the characteristics of an organism. The scientific name *Staphylococcus aureus* tells you the morphology and arrangement of the individual cells belonging to this bacterial genus (staphylococcus = spheres in clusters) and also tells you that *S. aureus* often grows in colonies with a golden color (“aureus”). Although scientific names are often descriptive occasionally these descriptions can be deceiving. For example, *Haemophilus influenza* is a bacterium (not a virus), and does not cause influenza.

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CHAPTER OVERVIEW

4: Introduction to Aseptic Techniques and Growth Media

[4.1: Introduction](#)

[4.2: Procedure](#)

[4.3: Results](#)

[4.4: Review Questions](#)

[4.5: Examples of Bacterial Growth Characteristics in Broths, Slants and Plates](#)

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4.1: Introduction

Learning Objectives

- Learn how to inoculate growth media using proper aseptic procedures
- Learn how to streak for single colonies
- Understand the uses of selective and differential growth media
- Determine the properties of some common bacterial types when grown on selective and differential growth media

Growth Media

To study bacteria and other microorganisms, it is necessary to grow them in controlled conditions in the laboratory. Growth media contain a variety of nutrients necessary to sustain the growth of microorganisms. There are two commonly used physical forms of growth media: liquid media and solid growth media. A liquid medium is called a **broth**. Solid growth media usually contains **agar**, which is a mixture of polysaccharides derived from red algae. It is used as a solidification agent because it (1) is not broken down by bacteria, (2) contains no nutrients that can be used by bacteria and (3) melts at high temperatures, and yet is solid at temperatures used for most bacterial growth. Solid growth media is used in the following forms: agar plates, agar slants, and agar deeps. To make agar deeps or agar slants, melted agar is poured into a test tube and then allowed to solidify vertically (agar deep), or at a slant (agar slant). Agar plates are made by pouring melted agar into a petri dish.

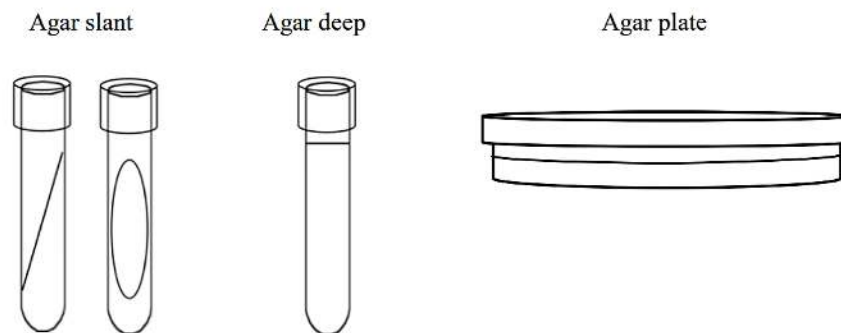


Figure 4.1.1: Solid growth media forms

Broths can be used to determine growth patterns in a liquid medium, and for certain types of inoculations and metabolic tests that you will be doing later in the semester. They are also the method of choice for growing large quantities of bacteria. Agar slants are commonly used to generate stocks of bacteria. Agar plates can be used to separate mixtures of bacteria and to observe colony characteristics of different species of bacteria (you will perform an experiment in this lab to illustrate this). Deeps are used for several different types of differential metabolic tests (e.g., the gelatinase test, which you will perform in Lab 5).

Growth media can be categorized based on their chemical constituents or the purpose for which they are used.

- **Complex growth media** contain ingredients whose exact chemical composition is unknown (e.g. blood, yeast extract, etc.).
- **Synthetic** (also called **chemically defined**) **growth media** are formulated to an exactly defined chemical composition.
- A **general-purpose growth medium** (e.g. tryptic soy agar (TSA) or Luria broth (LB)) is used to grow a wide variety of non-fastidious bacteria. This type of medium is often a complex growth medium.
- A **selective growth medium** contains chemicals that allow some types of bacteria to grow, while inhibiting the growth of other types. An example of a purely selective growth medium is PEA, phenylethyl alcohol agar, which allows Gram-positive bacteria to grow while inhibiting the growth of Gram-negative bacteria.
- A **differential growth medium** is formulated such that different types of bacteria will grow with different characteristics (e.g. colony color). An example of a differential growth medium is blood agar, which differentiates among bacteria based on their ability to break down red blood cells and hemoglobin. Blood agar is also a complex growth medium because it contains blood.

A growth medium can be both selective and differential. For example, EMB (eosin methylene blue agar) inhibits the growth of Gram-positive bacteria. Gram-negative bacteria that grow on this medium are differentiated based on their ability to ferment the sugars lactose and sucrose. (Note: the Gram staining procedure divides bacteria into 2 main groups: Gram-positive bacteria and Gram-negative bacteria, based on their cell wall structure. You will be doing Gram staining in the next lab period.)

Characteristics of Bacterial Growth

Even on general-purpose growth media, bacteria can exhibit characteristic growth patterns. On agar plates, bacteria grow in collections of cells called **colonies**. Each colony arises from a single bacterium or a few bacteria. Although individual cells are too small to be viewed, masses of cells can be observed. Colonies can have different forms, margins, elevations, and colors. Observing colony characteristics is one piece of information that microbiologists can use to identify unknown bacteria. Some examples of growth characteristics on different forms of growth media are shown at the end of the lab.

Aseptic Technique and Inoculation

Inoculation is the purposeful introduction of bacteria into a **sterile** growth medium. A material is sterile when it has no living organisms present; **contamination** is the presence of unwanted microorganisms. **Aseptic techniques** are practices that prevent the contamination of growth media.

When working in a microbiology laboratory, you must always remember that bacteria are present on all surfaces in the lab, as well as on your own hands and clothing. Aseptic techniques are designed to prevent the transfer of bacteria from the surrounding environment into a culture medium. These techniques require care and concentration. Pay attention to what you are doing at all times!

Aseptic techniques include the following practices:

1. Minimize the time that cultures and growth media are open to the environment.
2. Disinfect the work area before and after use.
3. Do not touch or breathe into the sterile culture media or the stock cultures.
4. Loops, needles, pipets, etc. should be sterilized before they are used.
5. **When working with tubes, the tube caps should not be placed on the tabletop; they should be held in your hand while inoculating.**
6. When removing the caps from test tubes, flame the lip of the test tube after the cap is removed. This heats the air inside the tube, so the air moves out of the tube, preventing contaminants from entering the tube.
7. Information about the use of the Bunsen burner can be found in the General Introduction in the Lab Manual.

General Procedure for inoculating media

Note

See figure 4.1.2

1. Sterilize an inoculating loop or needle in the flame of a Bunsen burner. The portion of the loop or needle that will contact the stock culture or the growth medium must turn bright orange for effective sterilization. For the most rapid sterilization, place the loop at the top of the inner blue cone of flame—this is where the temperature of the Bunsen burner is the hottest. Remove the loop from the flame after it is properly heated- keeping the loops in the flame for too long will eventually cause them to crack.
2. If you are picking a colony from a plate, cool the inoculating loop on agar that does not contain any bacterial colonies.
3. Pick a small amount of bacteria (you do not need much). If you are inoculating a tube of broth or an agar slant, remove the cap of the tube (do not set the cap down on the table) and flame the lip of the tube. Throughout the procedure, hold the tube at an angle to reduce the probability of particles entering the opening. Insert the loop into the tube and transfer bacteria to the growth medium. Be careful that only the sterilized part of the loop touches the tube or enters the growth medium.

4. Flame the lip of the test tube before replacing the cap.
5. Sterilize the inoculating loop again.

Streaking for single colonies

In the real world outside the laboratory, bacteria grow in communities made of many bacterial species. If you need to identify the types of bacteria present in environmental or medical samples, you must have a way to separate the different types and produce pure cultures. A **pure culture** contains a single bacterial species, whereas a **mixed culture** may contain many different types of bacteria. The process described in Procedure B (the streak plate method) describes the method that you will use to separate different types of bacteria in a mixture.

Inoculating a Plate from a Broth Culture

1. Sterilize the inoculating loop.
2. Remove the cap from the tube. Do NOT put the cap of the tube down on the lab bench—hold it in your hand.
3. Flame the lip of the tube.
4. Place sterile portion of inoculating loop into broth, then remove.
5. Flame the lip of the tube
6. Replace the cap.
7. Gently streak the surface of an agar plate with the inoculating loop.
8. Sterilize the inoculating loop.

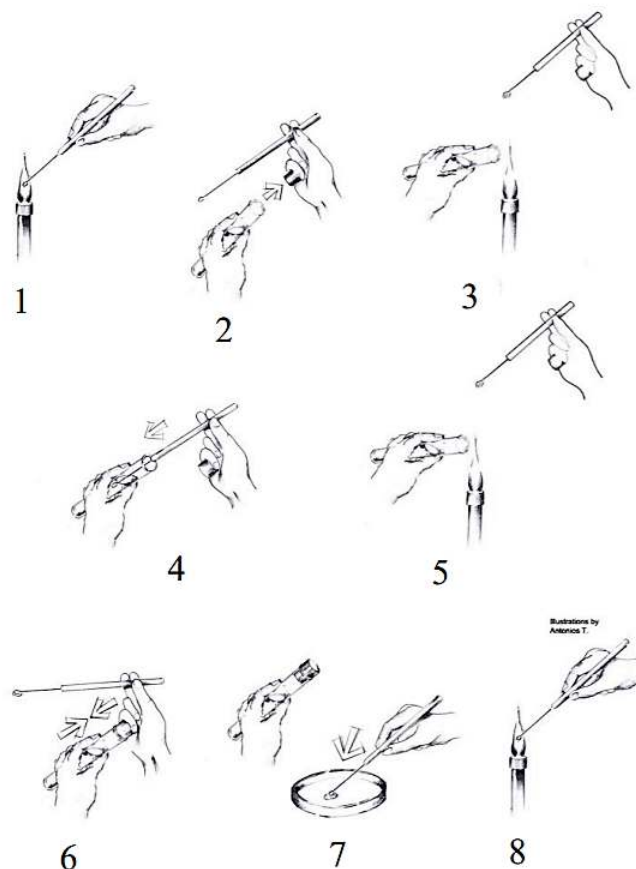


Figure 4.1.2: Procedure for inoculating a plate from broth culture

Notes about Labeling and Incubating Plates

1. Always label your plates/tubes BEFORE you do your inoculations. You can use Sharpies on the plates, but wax markers ONLY on tubes. When labeling tubes, label the tube itself—don't label the cap!
2. Make sure you label the **bottom** of the plates (the part of the plate that holds the agar).
3. Place plates inverted (upside down) for incubation. This prevents condensation from falling on the surface of the agar and disrupting the streaking pattern.

Media Used in This Lab Exercise

Note

See the Microbiology Review site for color pictures of media

Tryptic soy agar (TSA): General purpose complex growth medium.

Mannitol-salt agar (MSA): Differential and selective growth medium. This medium contains 7.5% NaCl, the carbohydrate mannitol and the pH indicator phenol red (yellow at pH 8.4). It is selective for staphylococci due to the high concentration of NaCl, and differentiates based on the ability to ferment mannitol. Staphylococci that ferment mannitol produce acidic byproducts that cause the phenol red to turn yellow. This produces a yellow halo in the medium around the bacterial growth.

Table 1: MS Agar

Selectivity	Interpretation	Identification
Growth	Growth Organism not inhibited by NaCl	E.g., <i>Staphylococcus</i> , <i>Micrococcus</i>
No Growth	Organism inhibited by NaCl	Not <i>Staphylococcus</i>
Differentiation		
Yellow Halo	Organism ferments mannitol	Probable <i>S. aureus</i>
No Yellow Halo	Organism does not ferment mannitol	<i>Staphylococcus</i> species (other than <i>S. aureus</i>); <i>Micrococcus</i> (yellow colonies)

Eosin-methylene blue agar (EMB): Differential and selective growth medium. This medium contains peptone, lactose, sucrose, and the dyes eosin Y and methylene blue. Gram-positive organisms are inhibited by the dyes, so this medium is selective for Gram-negative bacteria. The medium differentiates based on the ability to ferment lactose (and/or sucrose). Organisms that cannot ferment either of the sugars produce colorless colonies. Organisms that ferment the sugars with some acid production produce pink or purple colonies; organisms that ferment the sugars and produce large amounts of acid form colonies with a green metallic sheen. This medium is commonly used to detect the presence of fecal coliforms (like *E. coli*)—bacteria that grow in the intestines of warm-blooded animals. Fecal coliforms produce large amounts of acid when fermenting lactose and/or sucrose; non-fecal coliforms will produce less acid and appear as pink or purple colonies.

Table 2: EMB Agar

Result	Identification	Interpretation
No or poor growth	Organism inhibited by dyes	Organism is Gram-positive
Good growth	Organism not inhibited by dyes	Organism is Gram-negative
Colorless growth	Organism does not ferment sucrose or lactose	Non-coliform
Growth is pink and mucoid	Organism ferments lactose and/or sucrose with some acid production	Coliform bacteria
Growth is dark (purple to black with or without green metallic sheen)	Organism ferments lactose and/or sucrose, with large amounts of acid production	Possible fecal coliform (E. coli)

Key Terms

agar, broth, general purpose medium, selective medium, differential medium, colony, aseptic techniques, inoculation, streak-plate technique, contamination, sterile, pure culture, mixed culture

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4.2: Procedure

Make sure you follow aseptic procedures and LABEL everything carefully!

A. Observation of bacterial growth characteristics on selective and differential media

Each pair of students: 1 MSA and 1 EMB plate

Bacteria used: *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermis*, and *Pseudomonas aeruginosa*

Inoculation of selective and differential media

1. Divide the MS and EMB agar plates into 4 areas. Label each area with the type of bacteria that will be streaked there.
2. Use the inoculation loop to make a single streak of *S. aureus*, *E. coli*, *S. epidermis*, and *P. aeruginosa* in each of the 4 areas (see below).
3. Incubate the plate until the next lab period

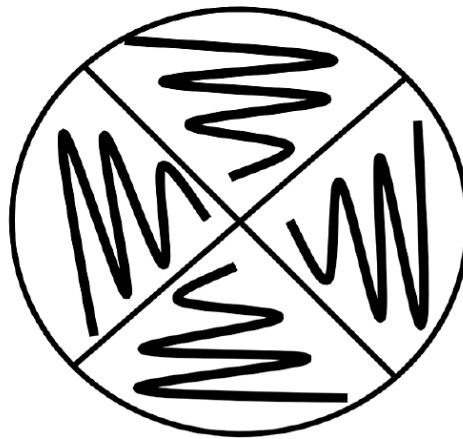


Figure 4.3.1: Step 2 of inoculation

B. Inoculation for single colony isolation

Each pair of students: 4 TSA plates

1. Sterilize your inoculating loop.
2. Use your loop obtain some bacteria from your sample.
3. Streak the bacteria on a small portion of the plate.
4. Sterilize your loop.
5. Cool the loop on an uninoculated part of the agar surface, and then pass it once through the already-streaked region of the plate. Streak another small portion of the plate.
6. Sterilize your loop. Cool the loop, then pass it once through the second streak, and make a third streak. Depending on the density of the original sample, single colonies will usually appear by the second or third streak.

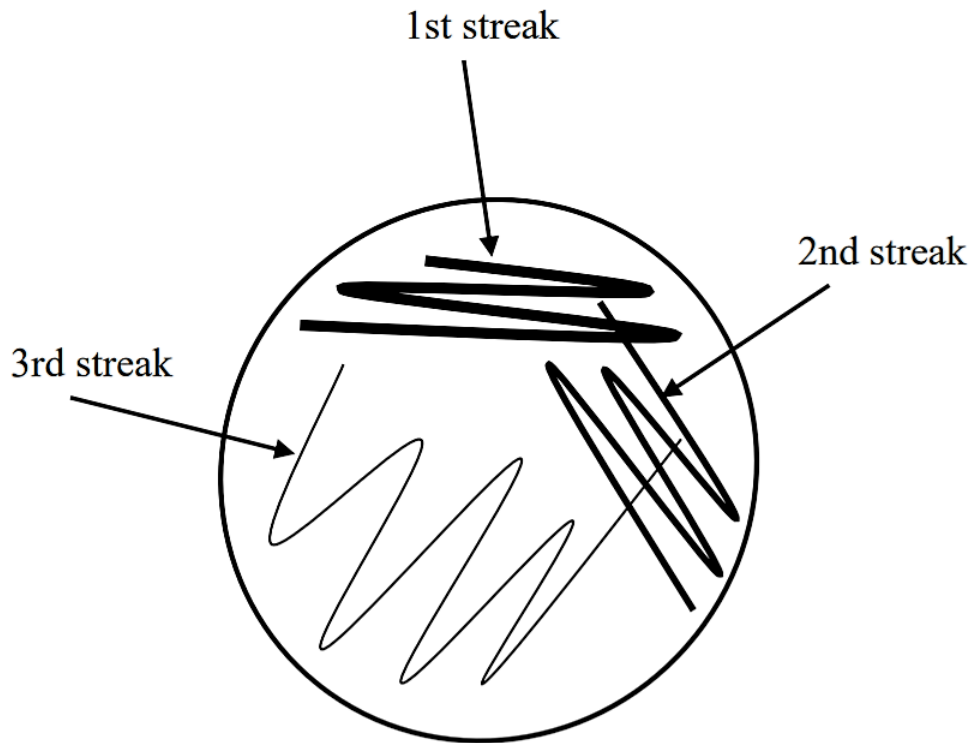


Figure 4.3.2: Streaking procedure

Use the procedure outlined above to streak *S. aureus*, *E. coli*, *S. epidermis*, and *P. aeruginosa* for single colonies onto individual TSA plates. Remember that you are trying to isolate single cells or a cluster of cells to form individual colonies. Do not pick up too much bacteria or spread it evenly all throughout the plate! Incubate until the next lab period.

C. Isolation of single colonies from mixed cultures using the streak plate method

Each pair of students: 1 MSA and 1 EMB plate

Mixed cultures: Unknown A and Unknown B

1. Streak Unknown A for single colonies on an MSA plate.
2. Streak Unknown B for single colonies on an EMB plate.
3. Incubate until the next lab period.

D. Culturing Microorganisms from Environmental Samples

Each pair of students: 1 MSA plate, 1 EMB plate, 1 TSA plate; 1 tube of sterile water; 1 sterile swab.

1. Pick one part of the environment that you would like to sample. Possibilities include: bottom of a shoe, floor, cell phone, sponge on a table, or the bottom of your handbag or backpack. Be inventive—pick an area you would find interesting! DO NOT take a sample from a person, the sample must be from an inanimate object. Communicate with your lab partners so that different environments are represented at your table.

My environmental sample is from: _____

2. Moisten a swab in the sterile water; before removing the swab, press it against the side of the tube to remove excess moisture.
3. Firmly swab the region you would like to sample.
4. Use the swab to inoculate the TSA plate, streaking the entire surface of the plate (see below). Use the same swab to streak the MS plate and the EMB plate.
5. Dispose of the swab in a designated plastic container at your lab table. (DO NOT throw swabs in the regular garbage!)

6. Seal your plates with parafilm, which will be demonstrated by your instructor.
7. Incubate the plates, being sure to invert the plates so that the agar side is on top. Your instructor will pull the plates when appropriate.

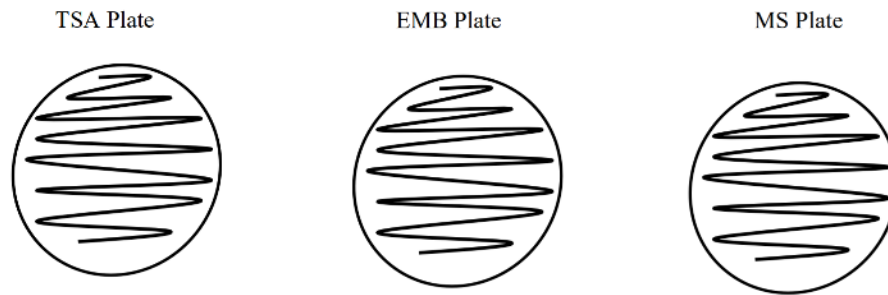


Figure 4.3.3: *Environmental sample plates*

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4.3: Results

Record the results of your experiments in the tables below.

A. Observation of growth characteristics on selective and differential media

Mannitol salt Agar			
Bacteria	Growth (+/-)	Describe growth characteristics	Interpretation
<i>S. aureus</i>			
<i>E. coli</i>			
<i>P. aeruginosa</i>			
<i>S. epidermis</i>			

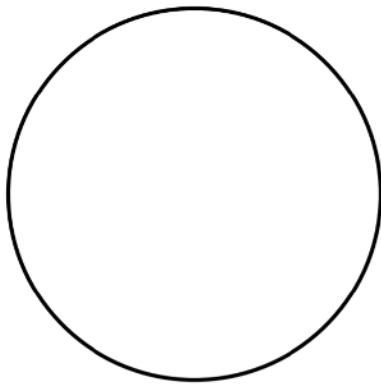
EMB Agar			
Bacteria	Growth (+/-)	Describe growth characteristics	Interpretation
<i>S. aureus</i>			
<i>E. coli</i>			
<i>P. aeruginosa</i>			
<i>S. epidermis</i>			

B. Inoculation for single colony isolation

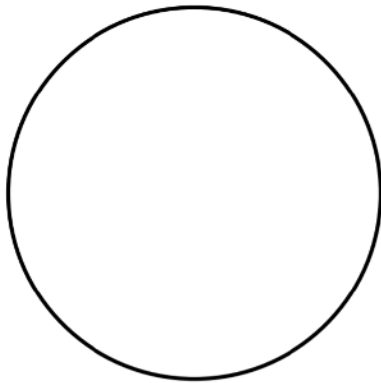
Tryptic Soy Agar		
Bacteria	Growth (+/-)	Describe growth characteristics
<i>S. aureus</i>		
<i>E. coli</i>		
<i>P. aeruginosa</i>		
<i>S. epidermis</i>		

C. Isolation of single colonies from mixed cultures

Unknown A: Describe growth characteristics on MSA and make a sketch in the circle below.



Unknown B: Describe growth characteristics on EMB and make a sketch in the circle below.



Based on your results, answer the following questions:

1. How effective was the streaking procedure in separating the different types of bacteria?
2. What conclusions do you have regarding the types of bacteria in the unknowns?

Unknown A

Unknown B

D. Environmental Sample

Record the results of your own environmental sample and the other sample at your bench in the two tables below.

Your environmental sample from _____

	Growth (+/-)	Describe growth characteristics
TSA		
EMB		
MSA		

Another group's environmental sample from _____

	Growth (+/-)	Describe growth characteristics
TSA		
EMB		
MSA		

Answer the following questions and provide evidence to support your answers.

1. Did you detect any *Staphylococcus* species in your environmental sample?
2. Were Gram-negative bacteria present?
3. Were there any coliforms present? Was *E. coli* present?
4. Was *S. aureus* present?
5. Was there evidence for organisms other than bacteria? (Ask your instructor.) Your instructor may ask you to save some of your environmental samples for further analysis.

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4.4: Review Questions

1. Based on your results and the information in the lab manual, fill in the table below.

	Predicted Gram Reaction	Ability to ferment lactose/sucrose	Ability to ferment mannitol	Coliform or Non-coliform
<i>S. aureus</i>				
<i>E. coli</i>				
<i>P. aeruginosa</i>				
<i>S. epidermis</i>				

2. What general type of growth medium would you use to:

- (a) grow one type of bacteria but inhibit the growth of another type?
- (b) discriminate between different types of bacteria?

3. Why is it necessary to sterilize the loop between streaks when streaking for single colonies?

4. Define and/or explain the use of the following:

- (a) synthetic medium
- (b) agar
- (c) broth

5. A bacterial species is inoculated on EMB agar.

- (a) The bacteria do not grow. Why?
- (b) If the bacteria ferment lactose, what would you expect to see?
- (c) The bacteria produce clear colonies. Why?

6. What medium would you use (TSA, EMB, MS) if you wanted to determine if a *Staphylococcus* isolate could ferment mannitol? Describe what you would see on this medium.

7. If you were testing water for the presence of fecal coliforms, what sort of medium would you use: TSA, EMB agar or MS agar? If fecal coliforms were present, what would their growth characteristics be on this medium?

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4.5: Examples of Bacterial Growth Characteristics in Broths, Slants and Plates

Even on general purpose growth media, bacteria can exhibit characteristic patterns of growth. Some examples are shown below. While these growth patterns are an important piece of information when identifying a bacterial species, they are not sufficient for a positive identification. Staining procedures and metabolic tests must be used for a definitive identification.

Growth Characteristics in Broths

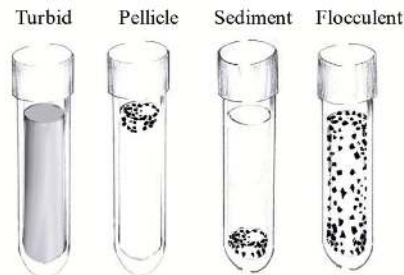


Figure 4.6.1: Bacterial growth in broths

Growth Characteristics on Slants

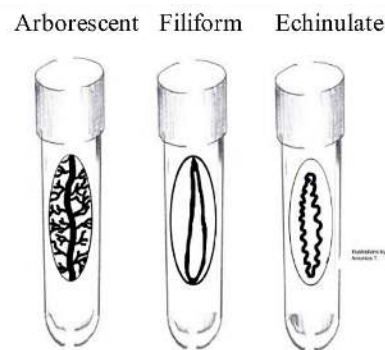


Figure 4.6.2: Bacterial growth on slants

Growth Characteristics on Plates

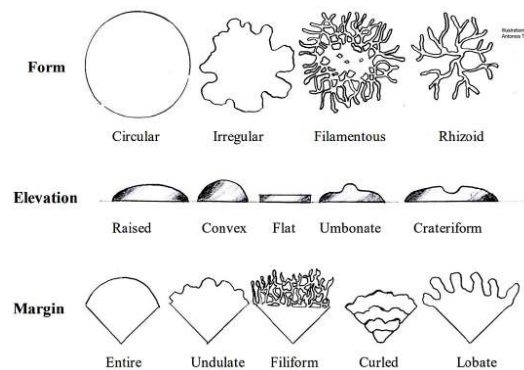


Figure 4.6.3: Bacterial growth on plates

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CHAPTER OVERVIEW

5: Quantifying bacterial cultures

[5.1: Determination of Bacterial Numbers](#)

[5.2: Introduction to Micropipetting](#)

[5.3: Serial Dilution](#)

[5.4: Procedures](#)

[5.5: Results](#)

[5.6: Dilution Worksheet and Problems](#)

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5.1: Determination of Bacterial Numbers

What is the Determination of Bacterial Numbers Approach and What is it Used for?



Figure 1: Professionals in government and industry measure the concentration of bacteria to maintain human safety and environmental health including: wastewater treatment (top left), food processing and food safety (top right), water recreation in oceans, lakes, ponds, rivers, and streams (bottom left), safety in swimming pools (bottom center), and safety of drinking water (bottom right). If treated wastewater still has too many bacteria, the water will undergo further treatment before being released into the environment. Foods that have unsafe levels of bacteria are disposed of and protocols enacted to fix the bacterial overgrowth in the facility. Natural waters that have unsafe levels of bacteria may be closed to recreation until they are safe again. Swimming pools may be closed if unsafe. Boil advisories are issued if drinking water has unsafe numbers of bacteria. This means that water used for drinking, cooking, and bathing must be boiled first to kill bacteria. These measures help to make sure that humans will not get sick and that the environment is kept safe.

Many microbiological studies require determining the concentration of viable bacterial cells within a sample. In addition to microbiological research, the concentration of viable bacterial cells is also important to examine to:

- make sure water is safe to drink
- make sure treated municipal wastewater is safe to release into the environment
- make sure water is safe to swim in (ocean, lakes, ponds, pools etc.)
- make sure food products are safe

Professionals in government and industry frequently test the safety of water and foods using the same type of approach described below to ascertain bacterial concentrations.

How does the Determination of Bacterial Numbers Approach Work?

The determination of bacterial numbers approach is used to answer one main question: what is the concentration of viable bacterial cells in this sample? This approach utilizes petri plates to conduct a standard plate count. The plate count will make it possible to determine cell concentrations, but it will take about 24 hours before we have those results.

Pairing the standard plate count technique with a faster method for estimating cell concentrations, absorbance measurements, can provide accurate cell concentrations within a couple of minutes (rather than waiting 24 hours for cultures to grow). This involves creating a standard curve using plate count results and absorbance readings. Once a standard curve is produced, the number of

bacteria in a sample can be determined using only absorbance readings and the standard curve (without the need to grow petri plate cultures for 24 hours).

Plate Count

Microbiologists use a technique called the **standard plate count** to estimate the population density of bacteria in a sample by plating a small and dilute portion of the sample and counting the number of bacteria colonies.

Note

A single bacterial colony originates from a single bacterial cell. When a single cell is deposited on a petri plate, it divides to form a colony that is visible with the naked eye. Therefore, when we count the number of isolated bacterial colonies on a petri plate, we are counting the number of cells present in that sample.

We use serial dilutions to create decreasing concentrations of the original sample that are then plated so that a plate will be created with a low enough number of bacteria that we can count individual colonies. From that number, we can calculate the original cell density in the broth. A turbid broth culture of bacteria can have millions or billions of bacterial cells in a single milliliter! If we transferred bacteria directly from that tube to a petri plate, it would be impossible to count colonies since there would be an overwhelming amount of growth on the petri plate (often called a **lawn** of bacteria).

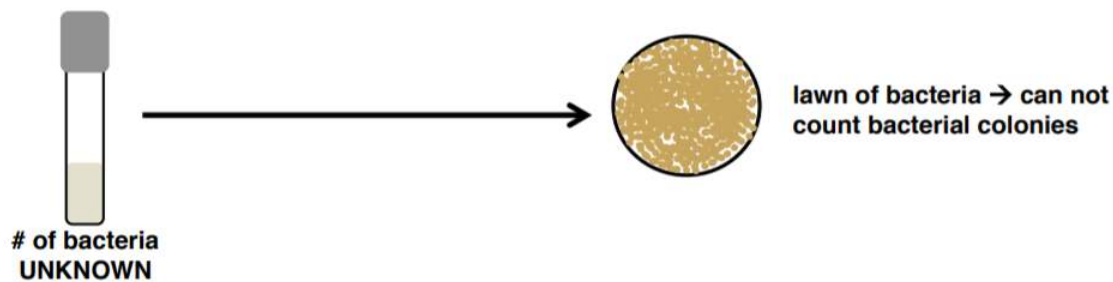


Figure 2: A bacterial culture can contain millions or billions of cells per milliliter! If even a small amount of that culture is plated directly onto a petri plate undiluted, it will form a "lawn" of bacteria where the colonies cannot be distinguished from each other since there are just so many of them growing together. This is why the standard plate count technique dilutes samples and conducts multiple dilutions to make sure that a countable number of bacterial colonies (25-250 colonies) is obtained on at least one of the petri plates.

Since we do not know how many bacteria are in a given sample or culture, we do not know how much we need to dilute the culture in order to grow a petri plate where the number of bacterial colonies is "countable" (we count a plate that has between 25-250 bacterial colonies). Therefore, we do a dilution series to create a number of plates, with a range of dilutions, in the hopes that we will produce a countable plate. The number of colonies we count on a petri plate enables us to calculate the **colony forming units** or **CFU**. CFU is a measure of the concentration of live, viable bacterial cells capable of reproducing when grown on a petri plate in cells per milliliter (cells/mL).

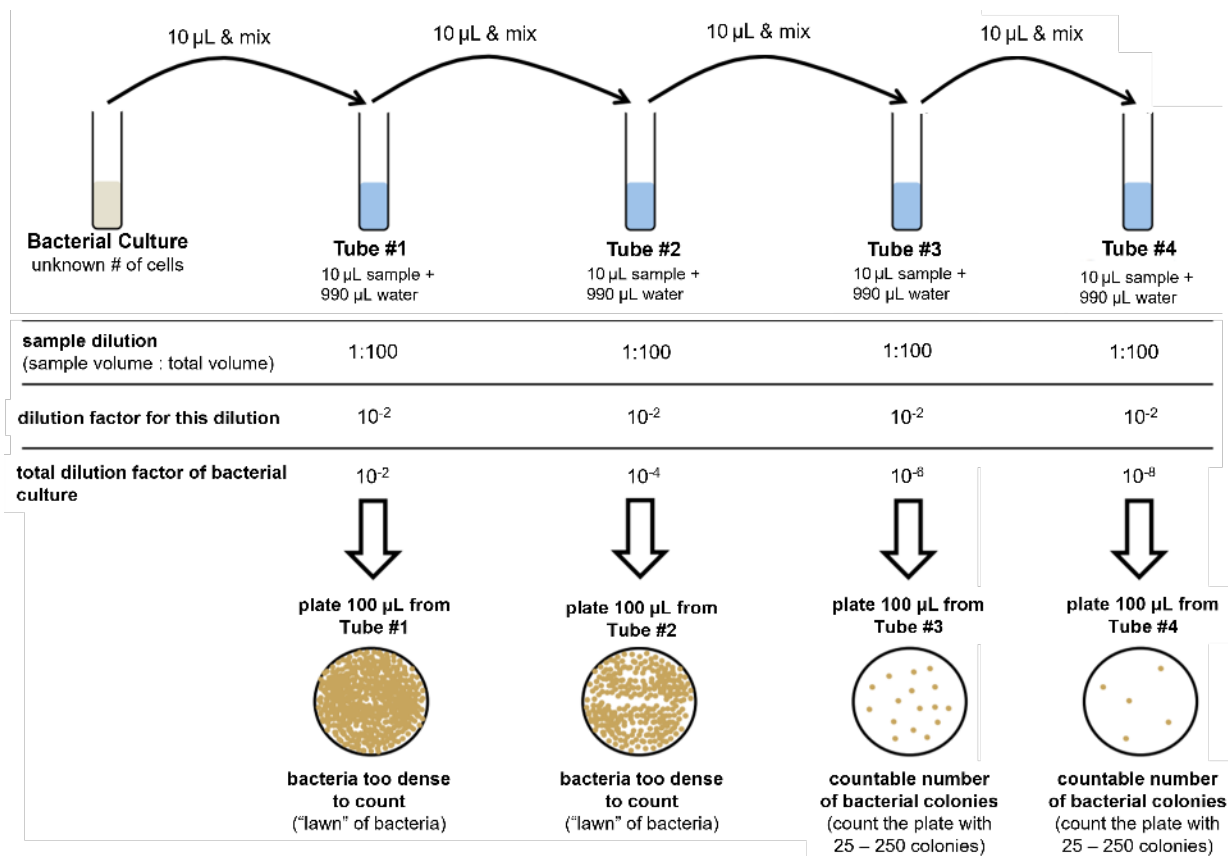


Figure 3: (Top) The process of diluting a bacterial culture in a dilution series is shown. In this process, 10 µL of the culture is diluted in 990 µL of water and mixed. This is a 1:100 dilution, or 10^{-2} dilution. A sample from this first dilution is plated onto a petri plate. The first dilution is further diluted and plated three more times in a similar manner. From the 1:100 dilution, 10 µL is removed and put into another 990 µL of water and mixed. This process is repeated to produce a total of four dilutions and four petri plates. After incubation of the petri plates, at least one plate should have countable colonies (between 25 and 250 colonies). From the counted colonies, the CFU can be calculated.

To Calculate CFU (cells/mL)

Step 1: Determine the concentration of cells in the diluted sample:

$$(\text{\# of colonies counted on the petri plate}) \div (\text{amount of diluted sample added to the petri plate in mL}) = \text{CFU in diluted sample (cells/mL)}$$

Note: 100 µL = 0.1 mL; 200 µL = 0.2 mL

Step 2: Determine the concentration of cells in the original sample:

$$(\text{CFU in diluted sample}) \div (\text{dilution of the petri plate counted}) = \text{CFU in original sample (cells/mL)}$$

✓ Example of CFU calculation

You conduct a standard plate count where 200 µL of each dilution is added to each petri plate. The petri plates diluted to 10^{-2} and 10^{-4} are lawns and cannot be counted. The 10^{-6} diluted petri plate results in 129 colonies. The 10^{-8} diluted petri plate has 12 colonies on it (so it is not used for the CFU calculation since it is not within 25-250 colonies). Calculate the CFU in cells/mL of the original sample based on these results.

Solution

- # of colonies counted = 129
- amount of diluted sample added to the petri plate in mL = 200 µL = 0.2 mL

- **dilution of the petri plate counted** = 10^{-6}

Step 1: Determine the concentration of cells in the diluted sample:

(# of colonies counted on the petri plate) ÷ (amount of diluted sample added to the petri plate in mL) = CFU in diluted sample (cells/mL)

(129 colonies) ÷ (0.2 mL diluted sample added to petri plate) = 645 cells/mL in the diluted sample

Step 2: Determine the concentration of cells in the original sample:

(CFU in diluted sample) ÷ (dilution of the petri plate counted) = CFU in original sample (cells/mL)

(645 cells/mL in diluted sample) ÷ (10^{-6} dilution of the petri plate counted) = 645,000,000 cells/mL in original sample

CFU = 645,000,000 cells/mL in the original sample

? Exercise 5.1

You conduct a standard plate count where 100 μ L of each dilution is added to each petri plate. The petri plates diluted to 10^{-2} and 10^{-4} and 10^{-6} are lawns and cannot be counted. The 10^{-8} diluted petri plate results in 211 colonies. Calculate the CFU in cells/mL of the original sample based on these results.

[Answer](#)

? Exercise 5.2

You conduct a standard plate count where 50 μ L of each dilution is added to each petri plate. The petri plates diluted to 10^{-2} is a lawn and cannot be counted. The 10^{-4} diluted petri plate results in 162 colonies. The 10^{-6} and 10^{-8} diluted petri plates both had less than 25 colonies on it (so it is not used for the CFU calculation since it is not within 25-250 colonies). Calculate the CFU in cells/mL of the original sample based on these results.

[Answer](#)

? Exercise 5.3

You conduct a standard plate count where 250 μ L of each dilution is added to each petri plate. The petri plates diluted to 10^{-2} and 10^{-4} are lawns and cannot be counted. The 10^{-6} diluted petri plate results in 89 colonies. The 10^{-8} diluted petri plate has 3 colonies on it (so it is not used for the CFU calculation since it is not within 25-250 colonies). Calculate the CFU in cells/mL of the original sample based on these results.

[Answer](#)

Absorbance Readings

Cells can absorb, transmit, and/or reflect light. **Spectrophotometry** is a technique where the amount of light a substance absorbs, or the amount of light that transmits through the sample, is measured.

A **spectrophotometer** is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through the sample solution.

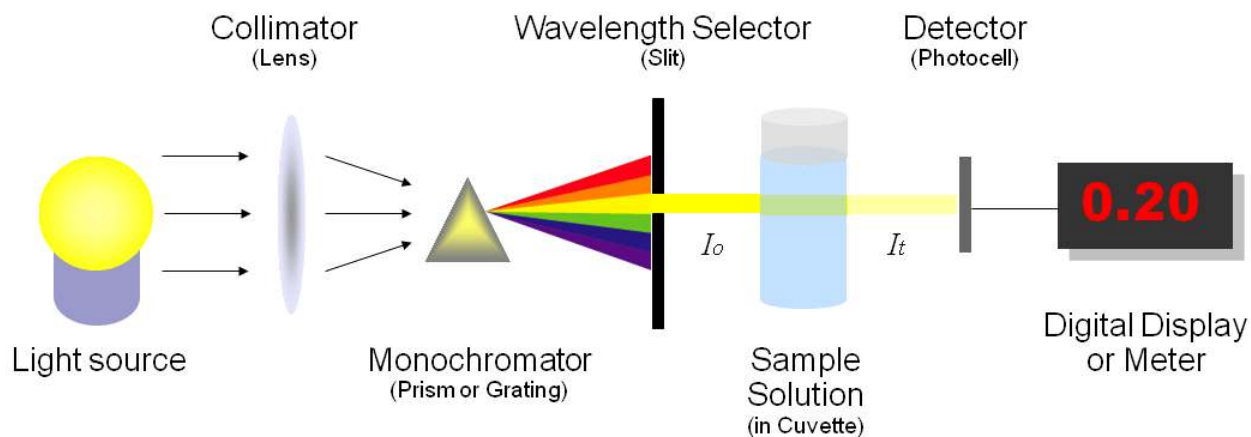


Figure 4: The diagram shows the components of a spectrophotometer. (Left) Light is emitted from a light source. (Second from the left) The wavelength of light is narrowed to a specific wavelength of light (accomplished by the monochromator) that is selected by the person using the spectrophotometer. (Second from the right) The light is passed through a sample in a container called a cuvette. The amount of light that reaches the other side of the sample is measured by a detector. (Right) The spectrophotometer displays the absorbance of light that is absorbed by the sample. The higher the absorbance, the more light did not pass through the sample. Therefore, the more cells in a sample, the higher the absorbance reading and the less light passing through the sample. A spectrophotometer can also be set to measure transmittance, which is the opposite of absorbance. Transmittance tells the person using a spectrophotometer how much light passes through the sample. Therefore, a sample with a lot of cells would have a lower transmittance reading than a sample with fewer cells.

Spectrophotometric (turbidimetric) analysis can be used to determine the quantity of cells in a sample based on the amount of light that is absorbed by the sample. It measures how turbid (cloudy) the solution is. When photons of light hit a cell, the light is absorbed, reflected, or diverted in a different direction. Therefore, the amount of light passing through a sample can be used to measure cell density in a sample.

Although using spectrophotometry to measure cell density in a sample can be quite effective, it will yield results that can be different from plate counts. For example, the standard plate count method is an indirect measurement of cell density and reveals information related only to live bacteria. The spectrophotometric analysis is based on turbidity and indirectly measures all bacteria (cell biomass), dead **and** alive.

Increased turbidity in a culture is another index of bacterial growth and cell numbers (biomass). By using a spectrophotometer, the amount of transmitted light decreases as the cell population increases. The transmitted light is converted to electrical energy, and this is indicated on a galvanometer. The reading, called **absorbance or optical density**, indirectly reflects the number of bacteria. This method is faster than the standard plate count but is limited because sensitivity is restricted to bacterial suspensions of 10^7 cells/mL or greater. Samples can be diluted to obtain accurate results when samples contain more than 10^7 cells/mL or more.

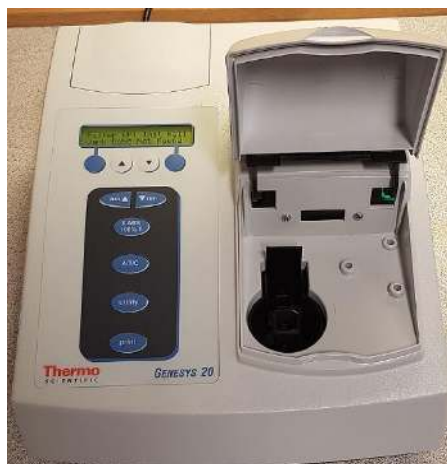


Figure 5: This image shows one type of spectrophotometer. The dark circle toward the right is where a sample is placed inside of the spectrophotometer. The lid is closed to allow light to pass through the sample and collect an absorbance or transmittance reading. The person using the spectrophotometer can select settings using the buttons on the left.

Putting it Together: Using Absorbance as a Quick Way to Determine Cell Concentrations

The CFU concentration determined in the standard plate count can be correlated with absorbance readings. This is done by setting up a graph with absorbance readings of different dilutions on one axis and cell concentrations of that culture at those dilutions on the other axis. Then the CFU is calculated for different dilutions and correlated with the corresponding absorbance readings at those same dilutions. These data points are plotted on a graph, and a line is drawn to best fit these data. This line, called a trend line or standard line, can be used to determine the cell concentrations of samples with unknown cell concentrations using absorbance only. This enables a microbiologist to quickly determine cell concentrations within a couple of minutes rather than taking the time and materials to conduct plate counts for every sample.

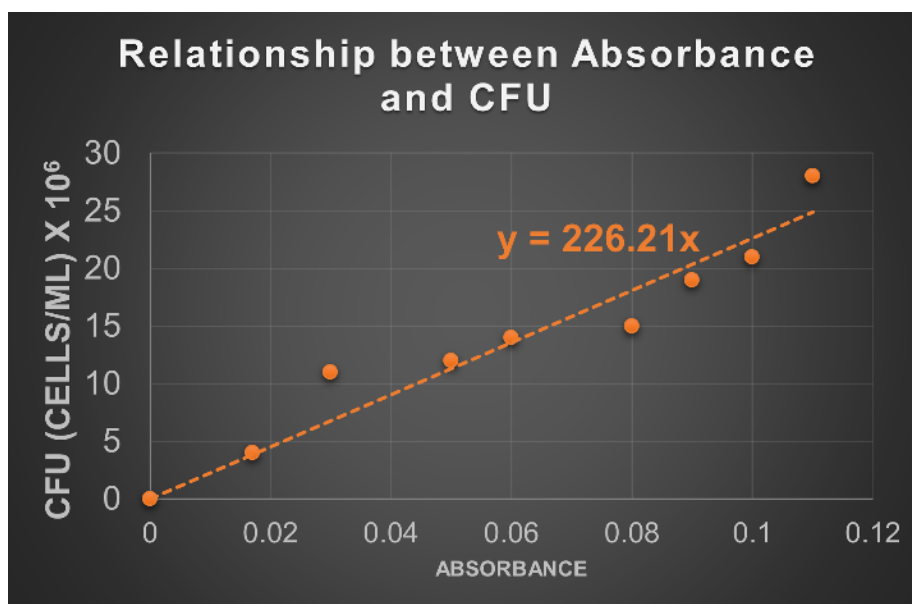


Figure 6: Absorbance data for a sample that has undergone serial dilutions is plotted against the CFU (based on standard plate count data) calculated at those dilutions. The result is a series of data points that can be used to plot a trend line. That trend line may be used to determine the CFU of samples without completing the standard plate count.

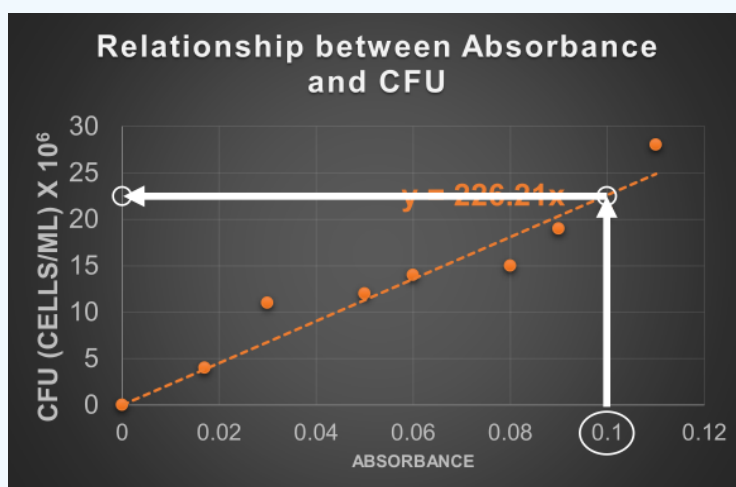
✓ Example of Using Position of Standard Line to Determine CFU of a Sample

You have a liquid sample containing the same species of bacteria used to make the standard line in the graph above. You want to find out the cell concentration of the sample in CFU without conducting a standard plate count because you need an answer right away (and not in 24-48 hours). You read the absorbance of the sample in the spectrophotometer. The absorbance reading is 0.1. Use the standard line above to determine the CFU.

Answer

The CFU at 0.1 absorbance is approximately 22.5×10^6 cells/mL or 2.3×10^7 cells/mL

Solution



1. Find the number corresponding to the absorbance on the graph's axis (in this case 0.1 on the x-axis).
2. On the graph, draw a straight line along the absorbance reading on the axis until it reaches the standard line. In this case, draw straight up from 0.1 until it hits the dotted standard line.
3. On the graph, draw a straight line from the point identified in step 2. to the other axis (in this case away toward the y-axis).
4. Where you intersect the other axis, determine the value of this point on the graph. This will be the approximate CFU at this absorbance. At 0.1 absorbance, the standard line is about halfway between 25 and 20 (22.5 is halfway between 25 and 20), but the unit given on the graph indicates these numbers should be multiplied by 10^6 . This is how 22.5×10^6 cells/mL was determined as the CFU at this absorbance. We may adjust how we present the number to 2.3×10^7 cells/mL.

? Exercise 5.4

You have a liquid sample containing the same species of bacteria used to make the standard line in **Figure 6**. The absorbance reading of the sample is 0.04. Determine the cell concentration of the sample in CFU without conducting a standard plate count.

Answer

An alternate way, and more accurate way, to determine the CFU from a standard line is to use the equation for the standard line. The equation for the standard line on the graph above is $y = 226.21x$. The way this graph is set up is with CFU on the y-axis, so the y in this equation is equal to CFU. The way this graph is set up is with absorbance on the x-axis, so the x in this equation is equal to absorbance. If we have an absorbance value, we just plug that number into the location where the x is and calculate what y is. y will be the CFU (but in this case because of the label on the y-axis, the answer will be $\times 10^6$)

✓ Example of Using Standard Line Equation to Determine CFU of a Sample

The absorbance of a sample is measured at 0.2. Use the equation from the standard line created from data in **Figure 6**.

Solution

- **Equation for standard line:** $y = 226.21x$
- **The absorbance (which is x):** 0.2
- **Set up the equation:** $y = (226.21) \times (0.2)$
- **Calculate y :** 45.242
- **Recall that the graph axis indicates y is $\times 10^6$, so the final answer is:** 45.242×10^6 cells/mL **or** 4.5×10^7 cells/mL

? Exercise 5.5

The absorbance of a sample is measured at 0.31. Use the equation from the standard line created from data in **Figure 6**.

Answer

? Exercise 5.6

The absorbance of a sample is measured at 0.18. Use the equation from the standard line created from data in **Figure 6**.

Answer

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5.2: Introduction to Micropipetting

When scientists need to transfer small volumes of liquid, they use a piece of equipment known as a micropipettor (or pipettor). There are a few different types of micropipettors based on the minimum and maximum volumes of liquid they are designed to transfer. Today you will learn how to use a micropipettor to a) transfer 1 ml (1000 μ l) volumes of liquid in the preparation of serial dilutions, and 2) transfer 0.1 ml (100 μ l) of these dilutions onto agar plates.

A. How to set the micropipettor for the appropriate volume:

The picture of the micropipettor shown below is similar to the one you will be using. As you can see, this micropipettor is designed to transfer volumes between 100 μ l -1000 μ l (this type of micropipettor is sometimes called a P1000). The display window reads “1000” when the pipettor is set to its maximum volume (1000 μ l or 1 ml). If we were using this same pipettor to transfer 500 μ l (or 0.5 ml) the window would read 500.

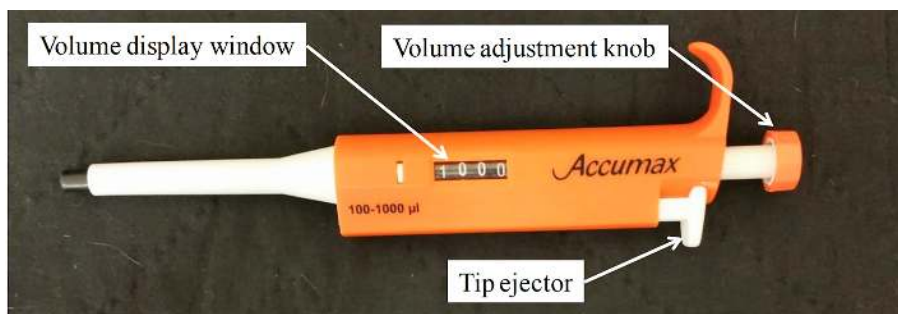


Figure 1: P1000 micropipettor

Similarly, the pipettor shown below (a P200) is designed to transfer volumes between 20- 200 μ l (0.02- 0.2 ml). It is set at 50 μ l —to pipet 100 μ l you would set the display to read ‘100’.



Figure 2: P200 micropipettor

The volume is set by turning the knob at the top of the pipettor until the correct volume is reached.

Note

Never try to turn the knob above the minimum or maximum volumes or you may damage the micropipettor!

B. How to attach a pipet tip:

After setting the appropriate volume, you will attach a pipet tip to the narrow end of the micropipettor. The P1000 uses blue tips; P200's use clear or yellow tips. The tips have been sterilized in the autoclave so as not to introduce any contaminating microbes into your sample. To pick up a tip:

1. Open the box of tips and insert the end of the pipettor into the end of a pipet tip.
2. Press down gently to ensure that the tip stays attached to the micropipettor.
3. Close the box to prevent the other tips from getting contaminated.

C. To transfer liquid using the pipettor:

Note

Follow these steps very carefully!

1. With your thumb, depress the plunger on the top of the pipettor until you first feel resistance.

Note

It is important not to go past the first stop. If you do, you will be picking up too much liquid!

2. While holding the plunger in the depressed position, place the pipet tip into the liquid you will be transferring.

Note

If you depress the liquid after placing the tip in the liquid, you will be blowing air into the liquid!

3. Slowly release the plunger to allow the tip to pick up the liquid.

Note

Be sure to keep the tip in the liquid the entire time so that you don't get air bubbles.

4. Place the tip into the tube (or plate) where you will be transferring the liquid to.
5. Slowly press the plunger down again until all the liquid leaves the pipet tip.
6. Withdraw the tip from the tube before releasing your thumb from the plunger.
7. Use the tip ejector to discard the tip in the container as instructed by your professor.

Note

Pipet tips NEVER get disposed of in the regular garbage.

Other important things to remember:

1. It is important not to let the pipet tip touch anything other than the liquid you are transferring.
2. Always keep the pipettor in the upright position when in use. If you tilt it too much the liquid in the tip will make contact with the pipettor and will no longer be sterile (this could also damage the pipettor).

Practice:

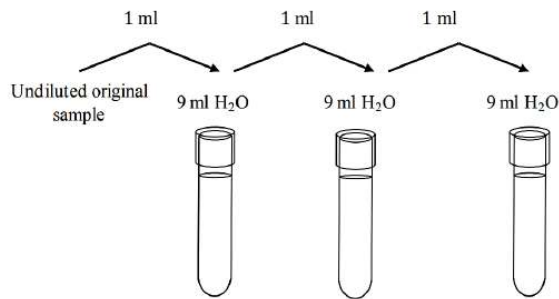
Set your P200 pipettor to 100 μ l and practice pipetting by transferring the liquid onto a piece of parafilm. Let your lab partners try as well. All droplets should be the same size (and the same size as your instructor's). Try transferring different volumes as instructed until you feel comfortable using the pipettor.

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5.3: Serial Dilution

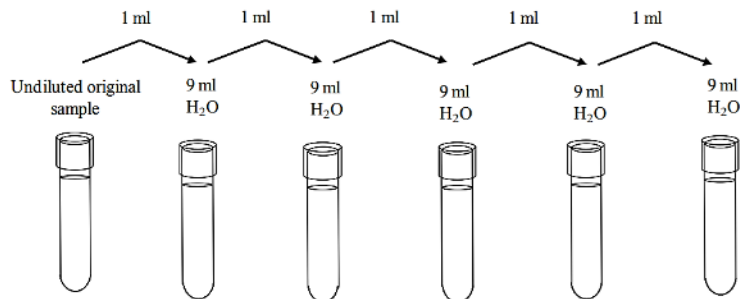
1. You have a urine sample from a patient that you suspect has a urinary tract infection. You make ten-fold dilutions of this sample as shown below, and then plate 0.5 ml (500 µl) of the last dilution on a TSA plate. There are 45 colonies on the plate. How many CFUs/ml were in the original urine sample?



2. You have received a sample from a sewage treatment plant, and have been asked to determine how many CFUs/ml are in this sample. You want to make a 1/100,000 fold dilution, but the smallest volume you can measure is 1.0 ml, and the tubes available to you only hold 10 ml. Explain/draw how you would do this!

3. You have a bacterial culture that you know has 650,000 bacteria/ml. You do serial dilutions to achieve a 1/10,000 dilution, and then plate 0.1 ml of each of these dilutions. How many colonies will you see at each dilution?

4. You do a series of dilutions as shown below, and you plate 1.0 ml of each dilution. Given the information below, fill in the number of colonies you would expect on each of the plates.



Number of colonies on each plate _____ _____ 446 _____ _____

5. You do serial dilutions on a water sample, and plate the dilutions on TSA plates. You count the colonies on each of the plates as follows: (Note: TMTC = too many to count)

Dilution	Number of CFUs
10^{-2}	TMTC
10^{-3}	TMTC
10^{-4}	352
10^{-5}	45
10^{-6}	6
10^{-7}	0

Based on these results, what is your estimate for the total number of CFUs/ml in the original sample?

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5.4: Procedures

Day 1

Today we will be practicing techniques used to quantify bacterial cultures. First, we will familiarize ourselves with micropipettes. Then we will practice dilutions and spectrophotometry using blue dye to simulate a bacterial culture.

Materials:

- Scale/balance Spectrophotometer
- Cuvettes
- micro pipettes and tips
- Water
- Blue food coloring
- Glass tubes

Using a micropipette

1. Tare (zero) your scale, then pipet and weight 1000 μl of water into a weigh dish. If your scale does not read between 0.9 and 1.1, repeat your attempts (taring between each measurement) until you are within that range.

Note

The density of water is 1 g/ml. When you weigh 1000 μl of water, you are weighing out 1 ml of water. Since 1 ml of water weighs 1 g, we can use the weight of the water to check the accuracy of our pipettes.

2. Remove the water from your weigh dish. Now we will record a series of measurements.
3. Tare your scale, then pipet and weight 1000 μl of water. Record your measurement in the table below.
4. Repeat pipetting 1000 μl of water (taring your scale between each attempt) and record each measurement in the table below. You must record a total of 10 attempts.

Record each measurement in the table below

Attempt #	Weight
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

Spectrophotometry

For our next experiment, we will be using blue dye to represent a cell culture. Blue dye absorbs light at $\sim 600\text{ nm}$, the same wavelength we use to measure cell density. Using a standard solution of blue dye, you will plot a curve of the dye's absorption at different dilutions. Based on this curve, you can predict the percentage of standard dye in an unknown solution.

1. Fill out the table below with the total percentage of standard dye in each solution, the volume (ul) of standard dye sample and water (ul) you would need to mix to achieve each dilution.
2. Dilute your standard sample of blue dye following the chart below.
3. Measure each dilution using the spectrophotometer and record the OD₆₀₀.

Table for cell culture

Dilution	Percent dye	Sample (ul)	Water (ul)	OD ₆₀₀
1.0 (no dilution)	100			
1/2	50			
1/3				
1/4				
1/5				
1/10				

4. Record which unknown solutions you received and record the OD₆₀₀ of your unknown solution.

Table to record unknown solutions

Unknown	OD ₆₀₀

Day 2

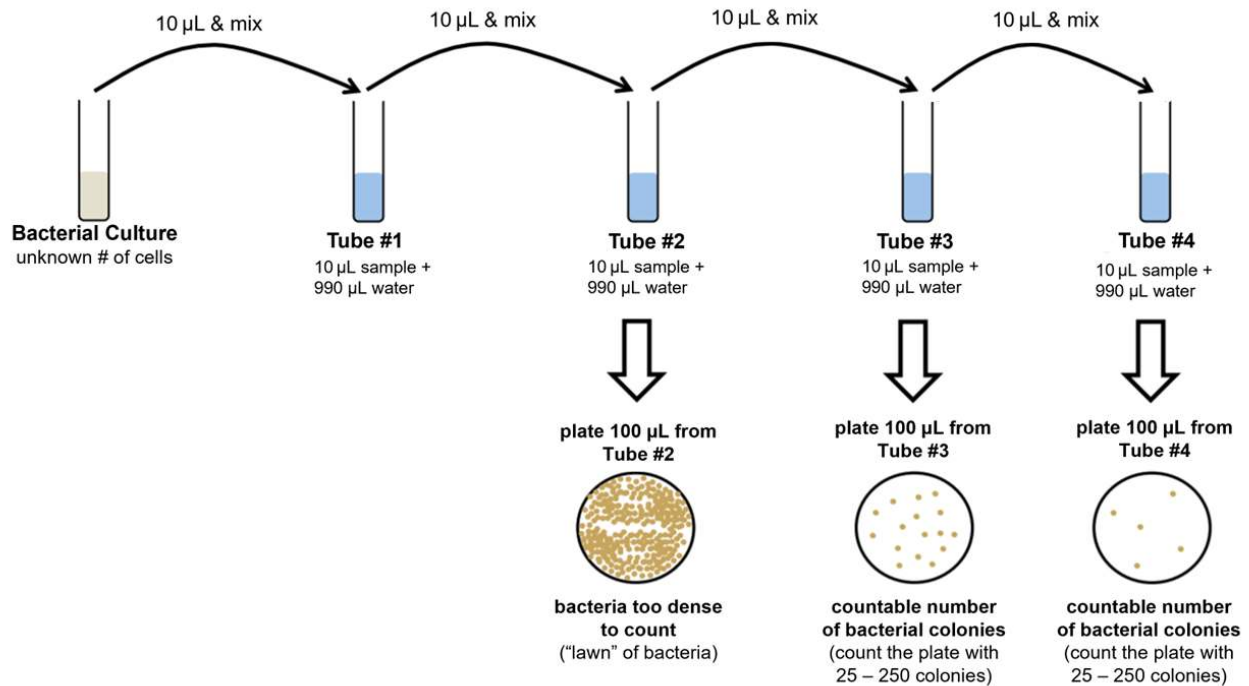
Today, we will use the micropipettes to perform serial dilutions of live cells. We will then plate the diluted cells on agar plates and incubate them overnight for quantification.

Materials:

- micro pipettes and tips
- 1.5 mL tubes
- Sterile Water
- *E. coli* K12 (overnight culture)
- 9 TSA plates per group

A. Spread plating

1. Dilute your *E. coli* sample according to the dilution chart below and spread plate the indicated samples in triplicate (three separate plates, each with 10 µl of sample from the indicated dilution).
2. Let your plates dry and incubate them inverted at 37°C



Day 3

Count your plates from Day 2 then record and analyze your results as described in the results section.

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5.5: Results

Definition: Percent error

Percent error calculates the difference between our observed and expected values as a percentage of the expected values.

$$\text{Percent Error} = \frac{(\text{Observed} - \text{Expected})}{\text{Expected}} \times 100$$

Percentage Error equation

1. Calculate the percent error for your pipettes using the average of the ten measurements you made with the scale. The **expected value** in our experiment was 1 g, since we expected 1000 µl of water to weigh 1 g. The **observed value** is the average of your ten measured weights, which is calculated by taking the sum of all the measurements divided by the number of measurements.

Calculated percent error _____

2. Discuss the sources of errors that likely affected your results.
3. For your blue dye measurements, graph the OD₆₀₀ readings (y-axis) against the percent dye in each solution (x-axis). Draw a best fit line for your plot.
4. Using your best fit line, estimate the percent dye for your unknown solution by finding the x coordinates where the best fit line intersects with the OD₆₀₀ reading you measured for your unknown.
5. Count the colonies for each of your plates and record your results in a table as the one below. Remember that the total dilution accounts for the number of µl you used to plate the sample!
6. Calculate the CFU/ml for each of your plates.

Example: Calculating CFU/ml

Jerrold and Sam are counting colonies for a plate that they pipet 100 µl of solution onto from a 1/1,000,000 dilution. Because 100 µl is 1/10 of a ml, they multiply the dilution of the sample (1/1,000,000) by 1/10 to calculate that the total dilution of that plate is 1/10,000,000 or 10⁻⁷.

Jerrold and Sam count 213 colonies from the plate with a 10⁻⁷ total dilution. To calculate CFU/ml they divide the number of colonies by the dilution. 213 divided by 10⁻⁷ is 213x10⁷ or 2.13x10⁹ or 2,130,000 colonies per milliliter.

Table:

Plate	Dilution	# of Colonies	CFU/ml
1			
2			
3			
4			
5			
6			
7			
8			
9			

7. Calculate the average CFU/ml.

8. Looking at your numbers would you say your variation (differences between values) is high or low? Name at least two possible sources of error when determining CFU/ml.

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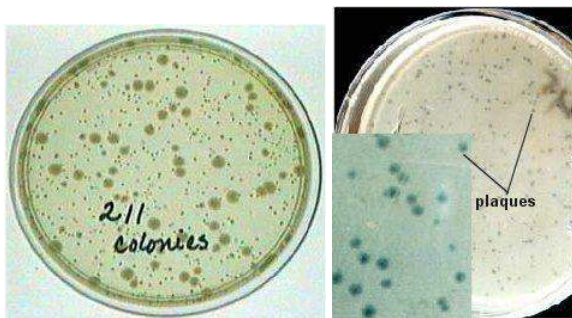
5.6: Dilution Worksheet and Problems

Objectives

- Understand how to quantify bacterial cells.
- Learn how to solve a dilution problem.

It is a common practice to determine microbial counts for both liquid and solid specimens--suspensions of *E. coli* in nutrient broth all the way to soil samples and hamburger meat. Most specimens have high enough numbers of microorganisms that the specimen has to be serially diluted to quantitate effectively. The following is a step-by-step procedure to working dilution problems, and includes some practice problems at the end.

The purpose can be the determination of bacterial, fungal, or viral counts (indirectly). This protocol is specific for bacterial counts (colony-forming units, CFUs), but can be modified for fungi (CFUs) and viruses (plaque-forming units (PFUs) for viral counts). A set of serial dilutions is made, a sample of each is placed into a liquefied agar medium, and the medium is poured into a petri dish. The agar solidifies, with the bacterial cells locked inside the agar. Colonies grow within the agar, as well as on top of the agar and below the agar (between the agar and the lower dish). The procedure described above produces a set of pour plates from many dilutions, but spread plates (sample spread on top of solidified agar) can be used also. The agar plate allows accurate counting of the microorganisms, resulting from the equal distribution across the agar plate. This cannot be done with a fluid solution since 1) one cannot identify purity of the specimen, and 2) there is no way to enumerate the cells in a liquid.



SOLVING DILUTION PROBLEMS

Note

colony count on agar plate

THE STANDARD FORMULA = _____
total dilution of tube (used to make plate for colony count) X amount plated

To work the problem, you need 3 values---a colony count from the pour or spread plates, a dilution factor for the dilution tube from which the countable agar plate comes, and the amount of the dilution that was plated on the agar plate.

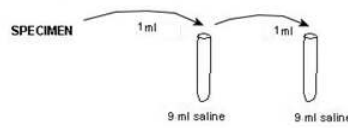
STEP 1: Determine the appropriate plate for counting

Look at all plates and find the one with 30-300 colonies (or plaques), preferably. Greater than 300 and less than 30 is a high degree of error. Air contaminants can contribute significantly to a really low count and a high count can be confounded by error in counting too many small colonies. Use the total dilution for the tube from where the plate count was obtained. If duplicate plates (with the same amount plated) have been made from one dilution, average the counts together.

STEP 2: Determine the total dilution for the dilution tubes

Dilution = amount of specimen transferred divided by the [amount of specimen transferred + amount already in tube].

Determine the dilution factor for each tube in the dilution series. Multiply the individual dilution of the tube X previous total dilution. **To calculate this dilution series:**



Note

amount of sample

Dilution factor for each tube in a set = _____

amount of sample + amount of diluent in tube

But after the first tube, each tube is a dilution of the previous dilution tube.

Note

Total dilution factor = previous dilution of tube X dilution of next container

✓ Example: FOR THE ABOVE DILUTION SERIES

1 ml added to 9ml = 1/10 for 1st tube

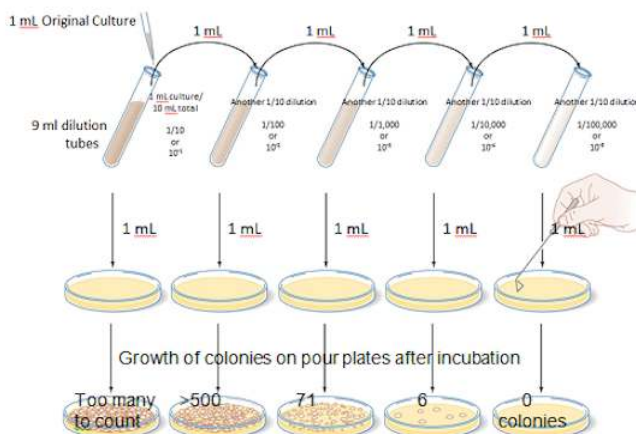
1ml added to 9ml = 1/10 for 2nd tube

previous dilution of 1/10 (1st tube) X 1/10 (2nd tube) = total dilution of 1/100 ($=10^{-2}=1/10^2$)

STEP 3: Determine the amount plated

The amount plated is the amount of dilution used to make the particular pour plate or spread plate.

There is nothing to calculate here: the value will be stated in the procedure, or it will be given in the problem.



? Solving the above problem

1. The countable plate is the one with 71 colonies.
2. The total dilution of 3rd tube from which above pour plate was made = $1/10 \times 1/10 \times 1/10 = 1/10^3$
3. The amount used to make that pour plate = 1ml

71 colonies

$$\frac{71}{1/10^3} = 71 \times 10^3 = 7.1 \times 10^4 \text{ (scientific notation) OR } 71,000/\text{ml}$$

Note: Rules for Scientific Notation

$$5.67 \times 10^5$$

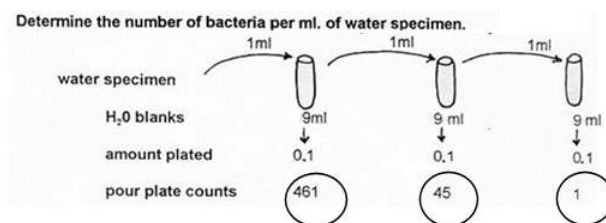
Coefficient Base Exponent

For a number to be in correct scientific notation, the following conditions must be true:

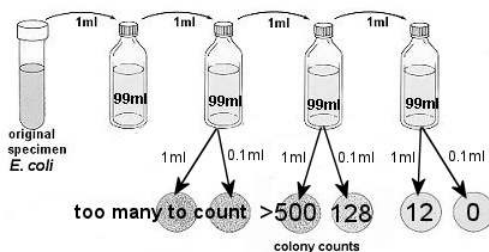
1. The **coefficient** must be greater than or equal to 1 and less than 10.
2. The base must be 10.
3. The exponent must show the number of decimal places that the decimal needs to be moved to change the number to standard notation. A negative exponent means that the decimal is moved to the left when changing to standard notation.

Questions

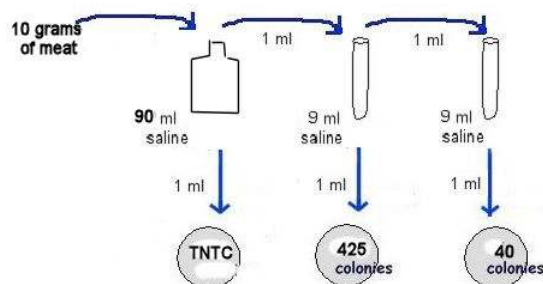
1. Determine the number of bacteria per ml. of water specimen.



2. Determine the number of bacterial cells per ml. in the original culture.



3. Determine the number of bacterial cells per gram of meat.



Contributors

- Jackie Reynolds, Professor of Biology ([Richland College](#))

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CHAPTER OVERVIEW

6: Preparation of Bacterial Smears and Introduction to Staining

[6.1: Introduction](#)

[6.2: Procedures](#)

[6.3: Results](#)

[6.4: Summary of Common Bacterial Staining Techniques](#)

[6.5: Review Questions](#)

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6.1: Introduction

Learning Objectives

- Learn the differences between simple staining and differential staining techniques.
- Learn how to prepare a bacterial smear from cultured organisms.
- Learn the differences between gram-positive and gram-negative bacteria.
- Learn how to perform the Gram stain procedure.
- Use microscopy to examine gram-stained cells.
- Learn about some special staining procedures and view examples of these under oil immersion.

Most types of cells do not have much natural pigment and are therefore difficult to see under the light microscope unless they are stained. Several types of stains are used to make bacterial cells more visible. In addition, specific staining techniques can be used to determine the cells' biochemical or structural properties, such as cell wall type and presence or absence of endospores. This type of information can help scientists identify and classify microorganisms and can be used by healthcare providers to diagnose the cause of a bacterial infection.

One type of staining procedure that can be used is the **simple stain**, in which only one stain is used, and all types of bacteria appear as the color of that stain when viewed under the microscope. Some stains commonly used for simple staining include crystal violet, safranin, and methylene blue. Simple stains can be used to determine a bacterial species' morphology and arrangement, but they do not give any additional information.

Scientists will often choose to perform a **differential stain**, as this allows them to gather additional information about the bacteria they are working with. Differential stains use more than one stain, and cells will have a different appearance based on their chemical or structural properties. Some examples of differential stains are the Gram stain, acid-fast stain, and endospore stain. In this lab you will learn how to prepare bacterial cells for staining and learn about the gram staining technique.

The Gram Stain

This very commonly used staining procedure was first developed by the Danish bacteriologist Hans Christian Gram in 1882 (published in 1884) while working with tissue samples from the lungs of patients who had died from pneumonia. Since then, the Gram stain procedure has been widely used by microbiologists everywhere to obtain important information about the bacterial species they are working with. Knowing the Gram reaction of a clinical isolate can help the health care professional make a diagnosis and choose the appropriate antibiotic for treatment.

Gram stain results reflect differences in cell wall composition. **Gram-positive** cells have thick layers of a peptidoglycan (a carbohydrate) in their cell walls; **Gram-negative** bacteria have very little. Gram-positive bacteria also have teichoic acids, whereas gram-negatives do not. Gram-negative cells have an outer membrane that resembles the phospholipid bilayer of the cell membrane. The outer membrane contains lipopolysaccharides (LPS), which are released as endotoxins when gram-negative cells die. This can be of concern to a person with an infection caused by a gram-negative organism.

Figure 6.1.1 shows the major differences between the gram-positive and gram-negative cell walls (also refer to your textbook for additional information). The differences in the cell wall composition are reflected in the way the cells react with the stains used in the Gram stain procedure.

Gram stains are best performed on fresh cultures—older cells may have damaged cell walls and not give the proper Gram reaction. Also, some species are known as **gram-variable**, and so both gram-positive and gram-negative reactions may be visible on your slide.

Although the vast majority of bacteria are either gram-positive or gram-negative, it is important to remember that not all bacteria can be stained with this procedure (for example, Mycoplasmas, which have no cell wall, stain poorly with the Gram stain).

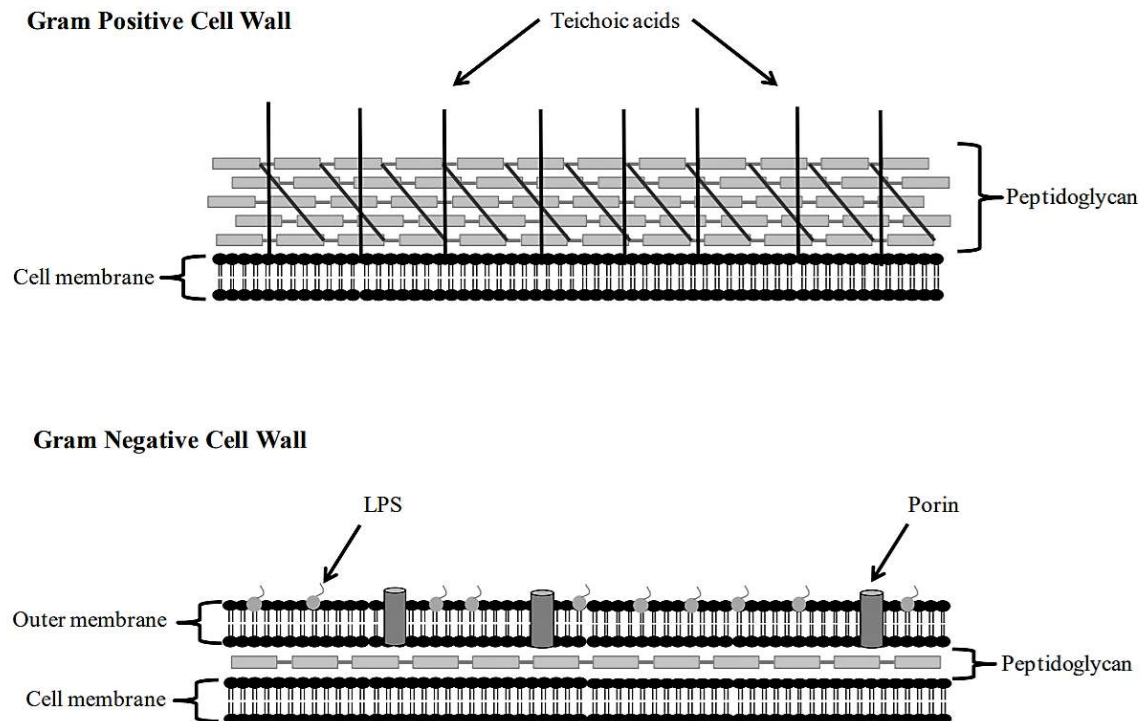


Figure 6.1.1: Gram positive and Gram negative cell walls

Special Stains

There are a variety of staining procedures used to identify specific external or internal structures that are not found in all bacterial species (see table at the end of this exercise for a comparison of staining procedures). You will do some of these staining procedures in the next lab (acid-fast staining and endospore staining). In today's lab, you will observe prepared slides of special stains: a capsule stain (*Klebsiella pneumoniae*), flagella stain (*Proteus vulgaris*), and spirochete stain (*Treponema pallidum*).

Capsule Stain

Some bacteria secrete a polysaccharide-rich structure external to the cell wall called a glycocalyx. If the glycocalyx is thin and loosely attached, it is called a slime layer; if it is thick and tightly bound to the cell, it is called a **capsule**. The glycocalyx can protect the cell from desiccation and can allow the cell to stick to surfaces like tissues in the body. They may also provide cells with protection against detection and phagocytosis by immune cells and contribute to the formation of a biofilm: in this way a glycocalyx can act as a virulence factor; (contributes to the ability of an organism to cause disease).

Capsules can be detected using a **negative staining** procedure in which the background (the slide) and the bacteria are stained, but the capsule is not stained. The capsule appears as a clear, unstained zone around the bacterial cell. Since capsules are destroyed by heat, the capsule staining procedure is done without heat-fixing the bacteria.

Silver Stain

Flagella (long whip-like structures used for bacterial motility) and some bacteria (e.g. **spirochetes**) are too thin to be observed with regular staining procedures. In these cases, a **silver stain** is used. Silver nitrate is applied to the bacteria along with a special mordant; the silver nitrate precipitates around the flagella or the thin bacteria, thus thickening them so they can be observed under the light microscope.

Key Terms

Gram stain, bacterial smear, simple stain, differential stain, Gram positive, Gram negative, Gram variable, capsule, spirochete, flagella, negative staining, silver stain

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6.2: Procedures

Preparation of a Bacterial Smear and Gram Staining

This semester you will be performing three staining procedures: Gram stain, acid-fast stain, and endospore stain. All three of these staining procedures begin with the preparation of a bacterial smear.

Materials

- Clean microscope slides
- Staining trays and newspaper
- Gram stain reagents: crystal violet, Gram's iodine, safranin, 95% ethanol
- Water bottle (for rinsing)
- Bacterial cultures (*determined by your professor*)

How to make a Bacterial Smear

1. Label a clean glass slide as demonstrated by your instructor.
2. Add a small drop of saline to the slide (you will usually put two bacteria on one microscope slide- Follow your instructor's specific instructions). This can be done by placing a drop of saline onto your inoculation loop and then transferring it to the slide. If you use the saline dropper directly on the slide, do not release a full drop.
3. With an inoculation loop or needle, pick up a small amount of bacteria. Mix it well with the saline and spread the mixture over a wider area of the slide.
 - Be careful not to have the two smears run into each other.
4. Air dry the bacterial specimen on the slide (slide warmers may also be used).
5. When slides are completely air-dry, heat fix the bacterial specimen by passing the slide slowly over the flame twice (your instructor will demonstrate this).
 - Heat fixing kills cells and adheres them to the slide.
 - Cells will be rinsed off the slides if they are not heat-fixed properly.
 - Be careful not to overheat the slides in this procedure

After heat-fixing is complete, you are ready to gram stain your slide.



Figure 6.2.1: Heat Fixing

Gram Staining Procedure

- For all steps in the gram staining procedure, add enough of the solution to cover the areas of the slide that have bacteria on them. You do not need to flood the entire slide.
- All staining should be done over a staining tray. Be sure to put a newspaper under the tray in case of spillage.
- Gloves should be worn while staining and removed before working with the microscope.

Gram Staining Procedure

STEPS	EXPLANATION
1. Add a few drops of crystal violet (primary stain) to the smear and let it sit for 60 seconds.	
2. Rinse the slide with water.	All cells are purple after this step. Stopping here would be a <u>simple stain</u> .
3. Add a few drops of Gram's iodine (mordant) to the smear and let it sit for 60 seconds.	Gram's iodine forms a complex with crystal violet
4. Rinse the slide with water.	All cells are purple after this step.
5. Decolorize with 95% ethanol : let the alcohol run over surface of the slide until no more crystal violet color comes out of the smear (time varies—no more than 5-10 seconds).	Gram-positive cells retain crystal violet and remain purple . Gram-negative cells lose crystal violet and are now colorless
6. Rinse with water.	Water rinse stops the decolorization process
7. Add a few drops of safranin (counterstain) and let it sit for 60 seconds.	Safranin is a pink/red dye
8. Rinse with water. Blot dry on bibulous paper. Be careful not to wipe off the bacteria.	Gram positive cells remain purple ; gram negative cells are now pink/red
9. Observe your slide under the microscope.	For each organism, determine morphology, arrangement and Gram reaction

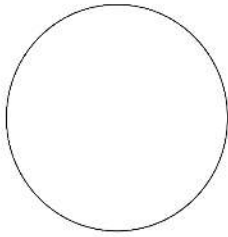
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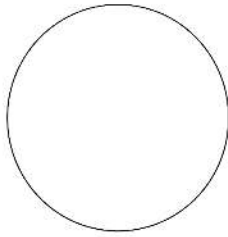
6.3: Results

Gram Stain Results

Draw sketches for each type of bacteria that you observe. Identify its morphology, arrangement, and Gram reaction.



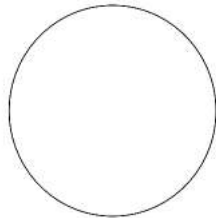
Species	_____
Morphology	_____
Arrangement	_____
Gram reaction	_____



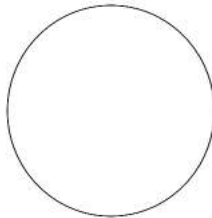
Species	_____
Morphology	_____
Arrangement	_____
Gram reaction	_____

Special Stain Results

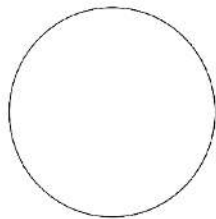
Observe the special stains set up at the demo microscopes at the back of the lab. Make sketches below.



Species	_____
Staining technique	_____
Comments:	_____ _____ _____



Species	_____
Staining technique	_____
Comments:	_____ _____ _____



Species	_____
Staining technique	_____
Comments:	_____ _____ _____

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6.4: Summary of Common Bacterial Staining Techniques

Simple Stains:

Crystal Violet, Methylene Blue, Safranin

- Used to provide color to otherwise transparent bacterial cells
- Can be used to determine cell size, morphology and arrangement

Gram Stain

Primary stain – crystal violet

Mordant – iodine; decolorizer- 95% Ethanol

Counterstain – Safranin

- Common differential stain
- Gram reaction (positive or negative) reflects cell wall properties
- Also used to determine cell size, morphology and arrangement

Acid-Fast Stain

Primary stain – Carbol fuchsin

Decolorizer – acid alcohol

Counterstain – Methylene blue

- A differential stain used to detect bacteria with mycolic acid cell walls (genera *Mycobacterium* and *Nocardia*)
- Developed to detect the bacterial species that causes tuberculosis
- Acid-fast organisms resist decolorization with acid-alcohol

Endospore Stain

Primary stain - Malachite green

Counterstain - safranin

- Endospores resist staining with basic stains
- Endospores stain with malachite green; vegetative cells stain with safranin

Capsule Stain (Negative staining)

Uses an acidic stain: (Congo red or Nigrosin) and a basic stain: (crystal violet or safranin)

- Negative stains are neither heat-fixed nor rinsed
- The background of the slide is stained by acidic stains (capsule remains unstained)
- The cells within the capsule are stained with Basic stains
- Examples of encapsulated cells: *Bacillus anthracis*, *Streptococcus pneumoniae*, and *Klebsiella pneumonia*

Flagella Stain

Silver nitrate

- Used to see bacterial flagella that are too slender to be seen with other staining techniques
- Silver nitrate makes flagella appear larger than they are
- Can be used to determine arrangement of flagella for identification.
 - Ex: *Proteus vulgaris* has peritrichous flagella

Spirochete stain

Silver nitrate

- Used to visualize slender spirochetes like *Treponema pallidum*

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6.5: Review Questions

1. Explain the major differences between the gram positive and gram negative cell wall.
 2. *Salmonella typhi* is a gram negative organism.
 - a. What color will it appear when simple stained with crystal violet?
 - b. What color would it be if it was gram stained correctly?
 3. Explain how bacterial cells would look in the gram staining procedure if the following mistakes were made:
 - a. Decolorizer left on too long
 - b. Decolorizer not left on long enough
 - c. Slide not heat-fixed before staining
 4. What is the difference between a simple stain and a differential stain?
 5. Explain why it is important to use only a small amount of bacteria when preparing a smear.
 6. What are the two things that are stained in a capsule stain? What is NOT stained in a capsule stain?
 7. Why would a health care provider be interested in knowing the gram reaction of a pathogenic bacterium?
-

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CHAPTER OVERVIEW

7: Metabolic Activities of Bacteria

[7.1: Introduction](#)

[7.2: Procedures](#)

[7.3: Results](#)

[7.4: Review Questions](#)

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7.1: Introduction

Objectives

- Learn how to use differential and selective media to identify bacterial species.
- Be able to perform the following biochemical tests and understand how they work: carbohydrate fermentation, gelatin hydrolysis, starch hydrolysis, casein hydrolysis, urea hydrolysis, citrate utilization, catalase activity, tryptophan hydrolysis (indole test).
- Be able to relate the results of the tests performed to the different types of metabolic activities carried out by individual bacterial species.

As you observed in Labs 3 and 4, staining procedures can be used to discriminate between bacteria based on morphology, arrangement, cell wall structure, and their possession of other structures associated with bacterial cells such as flagella, capsules, and spores. However, staining procedures are not sufficient to identify bacteria at the species level. For example, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *E. aerogenes*, and many other bacteria are all gram-negative rods and are indistinguishable by Gram staining. Therefore, additional methods are required to identify individual species.

Even though different bacterial species may appear identical under the microscope, they all have different genetic makeup (DNA), and therefore produce different types of enzymes that allow them to carry out a characteristic set of biochemical reactions. In other words, each bacterial species has a characteristic metabolism that can be used to distinguish them from other species, and therefore, unknown bacteria are often identified based on both their appearance under the microscope as well as their metabolic properties.

Examples of differences in metabolic properties were observed in Lab 2, when you inoculated *E. coli* and *P. aeruginosa* onto EMB agar. Each of these bacteria produced different colored colonies on this media, depending on their ability to ferment lactose. In addition to EMB and MS, there are many other types of differential media that can be used in the identification of bacteria.

When using differential media, it is always important to remember that reactions in these media can vary based on the incubation time, the incubation temperature, the number of bacteria in the initial inoculum, and how the medium is inoculated, among other variables. For example, an inoculum containing large amounts of bacteria might give a strong positive reaction, while one containing a very small number of bacteria might only produce a weakly positive, or perhaps a negative reaction. It is also important to remember that not all bacterial species will give definitive positive or negative results on these media. Since not all differential media will provide meaningful results for all bacterial species, it is important to choose the media carefully when identifying unknowns.

In many metabolic tests, end products are produced that change the pH of the medium. To measure this pH change, pH indicators (chemicals that change color depending on pH) are included in the medium. Some common pH indicators are phenol red, bromocresol purple, and bromothymol blue. Each pH indicator has a range of pH values over which it changes color (see below).

pH Indicator

pH Indicator			
phenol red	< pH 6.8 = yellow	pH 6.8 – 7.4 = red	pH >7.4 = pink/magenta
bromocresol purple	< pH 6.8 = yellow	> 6.8 pH = purple	
bromothymol blue	< pH 6.0 = yellow	pH 6.1 – 7.5 = green	pH >7.5 = blue

Note

Color pictures showing the results for all metabolic tests can be found on the Microbiology Review site on Blackboard.

A. Carbohydrate Fermentation

Fermentation is a metabolic process that some bacteria use to break down glucose when O₂ is not available. Fermentation includes the reactions of glycolysis (where a single molecule of glucose is broken down into 2 molecules of pyruvate), as well as

additional reactions that produce a variety of end products (acids, alcohols, gases). The end products are characteristic of individual bacterial species.

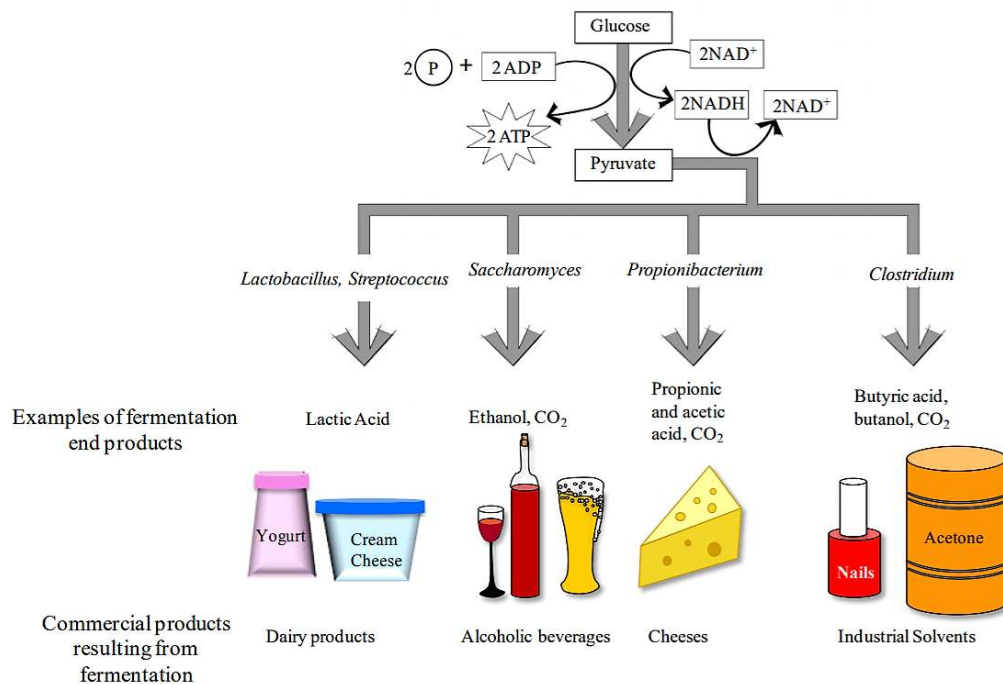


Figure 8.1.1: Carbohydrate fermentation

Although the ultimate substrate molecule for fermentation is always glucose, some bacteria use additional chemical reactions to convert other monosaccharides as well as disaccharides into glucose. Therefore, bacteria can be differentiated based on both their ability to ferment various carbohydrates as well as the end products that result from the fermentation process.

The medium used to test carbohydrate fermentation is a nutrient broth that contains a fermentable carbohydrate (usually a monosaccharide or a disaccharide), peptone (amino acids), as well as a pH indicator. The pH of the medium is adjusted to approximately 7.5, so it appears orange/red when using phenol red. If the carbohydrate in the medium is fermented and acidic end products are formed, a color change to yellow will result. Occasionally, bacteria will not ferment the carbohydrate, but instead will break down proteins, producing ammonia (NH₃) in the growth medium. In this case, the medium will become more alkaline.

Some bacteria will produce gases when fermenting a carbohydrate. To detect these gases, a **Durham tube** is used. This is a small, inverted tube that is placed within the larger glass tube containing the fermentation medium (see below). If gases (typically CO₂) are produced during the fermentation process, a bubble will form at the top of the Durham tube. If you see a bubble in the Durham tube, the medium will also be acidic.

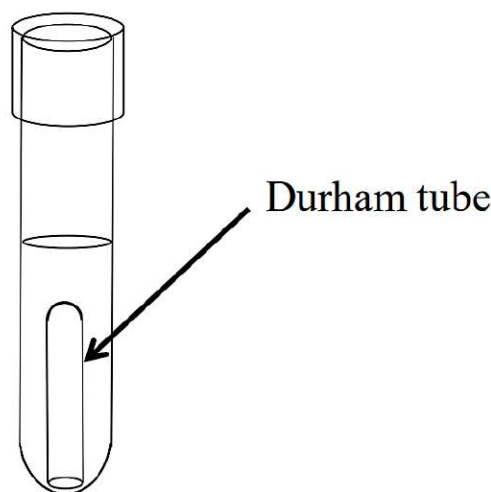
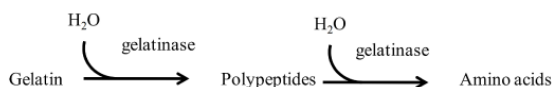


Figure 8.1.2: Durham tube

Carbohydrate fermentation media are often used to differentiate members of the family Enterobacteriaceae (e.g., *Escherichia coli*, *Enterobacter aerogenes*) from each other.

B. Gelatin Hydrolysis

Gelatin is a protein derived from the collagen found in connective tissue of bones, skin, and tendons. Robert Koch first used gelatin as a solidifying agent for growth media, but switched to agar when it was realized that gelatin does not stay solid at temperatures above 28°C and that some bacteria can chemically break it down. Some species of bacteria secrete an extracellular enzyme (exoenzyme) called **gelatinase** (a type of proteinase), that breaks gelatin down into its constituent polypeptides and amino acids. The small amino acids can then be brought into the bacterial cell. This is why agar is now used as the solidification agent in bacterial growth media. Since gelatinase breaks down connective tissue, it is sometimes called “spreading factor” and contributes to the virulence of a pathogen.



The presence of gelatinase can be detected by using nutrient gelatin deeps. This medium contains gelatin and nutrients for bacterial growth. A positive result is indicated by the gelatin becoming liquefied due to the enzyme activity. It is important to note that if the nutrient gelatin deeps are incubated at 37°C, then the medium **must be cooled** to below 25°C before a result can be recorded. A negative control is therefore very important in this experiment.

C. Starch Hydrolysis

Starch is a polymer of glucose that is too large to be transported into bacterial cells. For bacteria to be able to use the glucose in starch as an energy source, the macromolecule must be broken down extracellularly into smaller monosaccharide and disaccharide subunits. Some bacteria secrete the extracellular enzyme (exoenzyme) **amylase** that breaks down starch into monosaccharides and disaccharides (glucose and maltose). These smaller molecules can then be transported inside the bacterial cell.



Starch agar is used to determine if bacteria can produce the amylase enzyme. Bacteria that secrete amylase will break down the starch in the medium surrounding the bacterial colony. After the plate is inoculated and incubated, iodine is poured on the plate. The combination of iodine and starch results in the formation of a dark blue-black color. Regions of the agar where no starch is present (i.e., where starch has been broken down by amylase) will remain the color of the iodine (light yellow or gold).

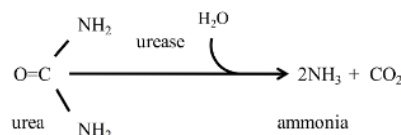
D. Casein Hydrolysis

Some bacteria secrete enzymes called proteinases that break down proteins. You have already read about one kind of proteinase—gelatinase. Milk contains large proteins called **caseins**. Some bacteria secrete **caseinases** that break down casein outside of the bacterial cell so the smaller products (e.g., amino acids) can be transported inside the cell and further metabolized.

Milk agar (which contains powdered milk) is used to detect the presence of bacterial caseinases. This medium is cloudy because when milk is mixed with agar, the casein forms a colloid through which light cannot pass. The presence of caseinases can be detected by observing a clearing in the agar around the bacterial growth, which indicates that the caseins have been broken down into transparent end products (amino acids and peptides), which are then taken up by the cells.

E. Urea Hydrolysis

Urea is a common waste product that results from the breakdown of proteins. Urea agar slants are used to test for the presence of a **urease** enzyme that is produced by some bacteria. Bacteria that produce ureases can break urea down into ammonia and carbon dioxide (see below). The ammonia that results from the breakdown of urea causes the medium of the slant to become more alkaline; this pH change is detected by the phenol red, which changes to a hot pink color. Organisms that do not break down urea may grow on the slant, but the slant will remain its original color, or will become more yellow (due to the production of acids).

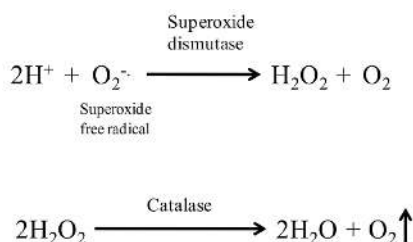


F. Citrate Utilization

Organisms that can survive using citrate as the sole source of carbon have a **citrate permease** enzyme that can transport citrate molecules into the cell. The citrate is then made into pyruvate, which can be converted into a variety of different products. **Simmon's citrate** is a chemically defined medium that contains sodium citrate as the sole carbon source as well as the pH indicator Bromothymol blue. Bacteria that can grow on this medium (i.e., that can survive on citrate as the sole source of carbon) produce alkaline byproducts that will change the medium from green (neutral pH) to blue (alkaline pH).

G. Catalase Activity

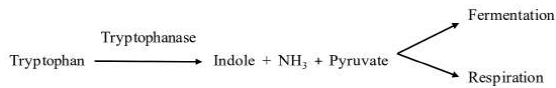
Byproducts of aerobic metabolism include two toxic compounds: superoxide free radicals (O_2^-) and hydrogen peroxide (H_2O_2). To remove these compounds, cells produce enzymes to break them down. Cells can convert superoxide free radicals to hydrogen peroxide by using the enzyme superoxide dismutase (SOD); **catalase** breaks down hydrogen peroxide into water and oxygen.



A simple test to determine if bacteria produce catalase is to add hydrogen peroxide to bacteria on an agar slant or to bacteria spread on a slide. If catalase is present, the hydrogen peroxide will be broken down into water and oxygen gas, resulting in the production of bubbles. This test does not require any special type of medium. However, it should never be performed on organisms that have been grown on blood agar (a medium that contains blood). This is because there is a catalase activity in blood that would produce a false positive result. Most aerobic and facultatively anaerobic organisms produce SOD and catalase (note: some species use peroxidase rather than catalase to break down hydrogen peroxide). Obligate anaerobes lack these enzymes, which is why they cannot survive in an atmosphere containing oxygen.

H. Tryptophan Hydrolysis

The Indole Test Tryptophan is an amino acid found in most proteins. Some bacteria produce **tryptophanase**, an enzyme that breaks tryptophan down into **indole**, ammonia and pyruvate (see below). The pyruvate and ammonia are converted into other molecules, but the indole accumulates, and thus can be detected in the media.



The presence of indole indicates that an organism produces the enzyme tryptophanase. Indole can be detected using a chemical known as **Kovac's reagent**. We will be using a **BBL dry slide** to detect indole. This is a disposable slide that employs a dry reagent slide impregnated with Kovac's reagent. The slide is divided into four large segments to enable multiple tests on one slide. The presence of indole will cause a pink color to develop on the dry-slide; no color change will occur if no indole is detected.

Note

For the indole test to be performed, the organism must be grown using a medium containing tryptophan.

Indole + Kovac's reagent + HCl + Amyl Alcohol → Rosindole dye (pink-red)

Key Terms

fermentation, amylase, gelatinase, caseinase, urease, citrate permease, catalase, indole, tryptophanase, Kovac's reagent, BBL Dry Slide, agar slant (locate butt and slant), Durham tube, Simmon's citrate

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7.2: Procedures

Make sure you follow aseptic procedures and label everything carefully!

Use the inoculation method indicated for each type of medium—these methods may differ. Make sure to thoroughly sterilize your loop or needle between inoculations to ensure that you are only introducing one bacterial species into your medium. If a medium is inoculated with more than one kind of bacterium, a positive result cannot be attributed to a single bacterial species. Be sure to use the correct bacterial species for each test. Follow directions carefully so that you do not waste media.

For comparison purposes, you will be provided with negative controls (media that have not been inoculated) in the next lab when you are analyzing your results.

Note

All media that you inoculate today will be incubated until the next lab, when you will analyze your results.

A. Carbohydrate Fermentation

Each student: 1 tube each of the following broths: Lactose + phenol red (green cap), Sucrose + phenol red (yellow cap), Glucose + phenol red (red cap)

Instructions: Choose 1 of the following bacteria: *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis*, or *Streptococcus faecalis* (each person at the table should choose a different species)

Inoculate the 3 types of fermentation broth with your chosen bacteria. Prior to inoculating the broths, make note of any small bubbles that might be present in the Durham tubes, so these are not read as evidence of gas formation during fermentation.

B. Gelatin Hydrolysis

Each student: 1 nutrient gelatin deep

Instructions: Choose 1 of the following bacteria: *Staphylococcus aureus*, *Serratia marcescens*, *Streptococcus faecalis*, or *Bacillus subtilis*

Use an **inoculation needle (not a loop)** to obtain some bacteria from your stock culture. Inoculate the gelatin deep by using the inoculating needle to stab ~3/4 of the way down into the nutrient gelatin and then drawing the needle straight back up.

C. Starch Hydrolysis

Each student: 1 starch agar plate

Instructions: Use all of the following bacteria: *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis*

Divide your starch agar plate into 4 areas using a wax pencil or a Sharpie marker. Make sure you draw on the bottom of the petri dish (the part that contains the agar) rather than on the lid. Label the plate to indicate which bacterium will be inoculated into each area. One area is left as a negative control.

Using a loop, you will do spot inoculations of each bacterial species in the areas. A spot inoculation is used to ensure that a large amount of bacteria will grow at a single location and produce a concentrated amount of amylase. The spot inoculation technique is shown below.

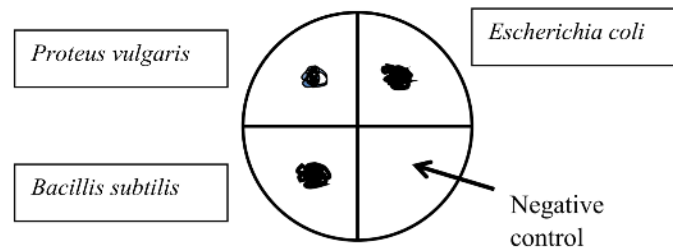


Figure 7.2.1: Spot inoculation technique for starch hydrolysis

D. Casein Hydrolysis

Each student: 1 milk agar plate

Instructions: Use both of these bacteria: *Bacillus subtilis* and *Enterobacter aerogenes*

Divide your milk agar plate into 3 areas using a wax pencil or a Sharpie marker. Label the plate to indicate which bacterium will be inoculated into each area. One area is left as a negative control. Using a loop, you will do spot inoculations of each bacterial species in the areas, just as you did on the starch agar plate.

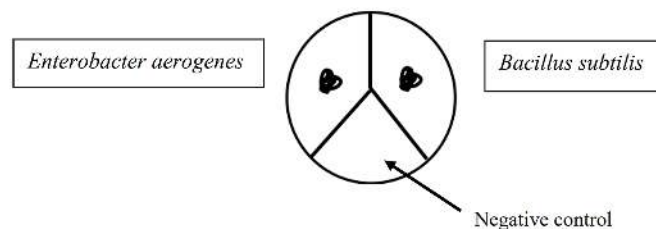


Figure 7.2.2: Spot inoculation technique for casein hydrolysis

E. Urea Hydrolysis

Each pair of students: 1 urea slant

Instructions: Choose one of these bacteria: *Escherichia coli* or *Proteus vulgaris*

To inoculate the urea slant, use a loop to obtain some bacteria from the culture, and then carefully streak the surface of the slant. Do not stab down into the slant's interior. Replace the cap on the slant, but leave the cap somewhat loose. Wrap in the tube in aluminum foil.

F. Citrate Utilization

Each student: 1 Simmon's citrate slant

Instructions: Choose one of these bacteria: *Escherichia coli* or *Serratia marcescens*

Using an inoculation needle (not a loop), inoculate the Simmon's citrate by first stabbing the needle into the **butt of the agar slant** (see below) and then streaking the surface of the **slant** before you pull the needle out of the tube.

Stab and Streak Method

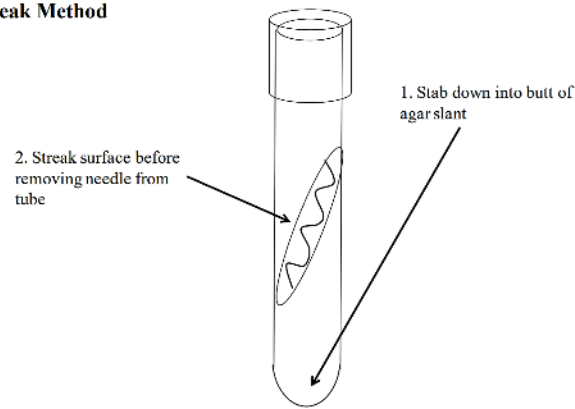


Figure 7.2.3: Procedure to inoculate Simmon's citrate

G. Catalase Activity

Each student: 1 TSA slant

Instructions: Choose one of these bacteria: *Staphylococcus aureus* or *Streptococcus faecalis*

Using a loop, inoculate the surface of the TSA slant. Make sure to use a heavy inoculum.

H. Tryptophan Hydrolysis

Each student: 1 tryptone broth (Note: this medium contains tryptophan)

Instructions: Choose one of these bacteria: *Escherichia coli* or *Enterobacter aerogenes*

Inoculate the medium with your chosen bacteria. Make sure to use a heavy inoculum.

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7.3: Results

Record your results for the metabolic tests in the tables on the following pages. At the end of this lab there is also a table where you can record test results for all of the organisms that will be on your first practical exam. It is important to obtain results all of the organisms in the table, as you will need this information to identify your unknown organisms for your midterm practical.

A. Carbohydrate Fermentation

Observe the results of your own carbohydrate fermentation test, as well as the tests done by your table partners. You can compare your inoculated tubes with the negative controls in the front of the class at the instructor's table. Record the results in the table below.

The following convention is used when noting the results of fermentation experiments.

A = acid

G = gas

AG means that both acid and gas are present

If neither acid nor gas is present, you can write "negative"

Carbohydrate Fermentation

Bacteria	Glucose	Lactose	Sucrose
<i>Proteus vulgaris</i>			
<i>Escherichia coli</i>			
<i>Bacillus subtilis</i>			
<i>Streptococcus faecalis</i>			

B. Gelatin Hydrolysis

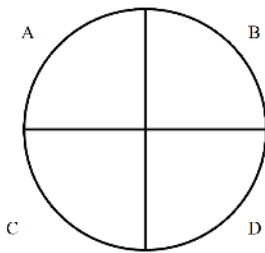
Observe your gelatin deeps and those of your table partners. Record your results in the table below.

Gelatin Hydrolysis

Bacteria	Liquid or solid	Result (+/-)
<i>Staphylococcus aureus</i>		
<i>Serratia marcescens</i>		
<i>Streptococcus faecalis</i>		
<i>Bacillus subtilis</i>		

C. Starch Hydrolysis

Add enough Gram's iodine to the plate to ensure that the entire areas of growth and the agar surrounding it are covered (you should also add iodine to the negative control area). Once you have added the iodine, make sure you do not tip the plate too much! Observe the appearance of your plate. Record your results in the table below.

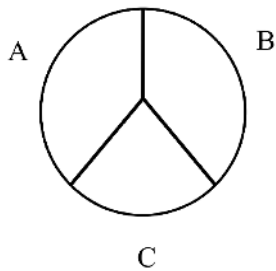


Starch Hydrolysis

Bacteria	Color change? (Y/N)	Starch present or absent?	Presence (+) or absence (-) of amylase
A. <i>Proteus vulgaris</i>			
B. <i>Escherichia coli</i>			
C. <i>Bacillus subtilis</i>			
D. Negative control			

D. Casein Hydrolysis

Observe your casein plate. It is helpful to hold it up to the light so you can detect the clear zones. Make a drawing of your results in the circle below. Indicate the location of the bacterial growth and draw any clear zones that are present. Record your results in the table below.



Casein Hydrolysis

Bacteria	Clear zone?	Result (+/-)
A. <i>Enterobacter aerogenes</i>		
B. <i>Bacillus subtilis</i>		
C. Negative control		

E. Urea Hydrolysis

Observe your urea slants. Record the results in the table below.

Urea Hydrolysis

Bacteria	Color	Result (+/-)
<i>Escherichia coli</i>		
<i>Proteus vulgaris</i>		

If the urea slant is pink, what can you say about the pH of the medium?_____ This pH change is due to what molecule? _____

F. Citrate Utilization

Observe your Simmon's citrate slants. Record the results in the table below.

Citrate Utilization

Bacteria	Color	Growth?	Result (+/-)
<i>Escherichia coli</i>			
<i>Serratia marcescens</i>			

G. Catalase Activity

Add a dropper full of H₂O₂ to the surface of the slant. Record your results below.

Catalase Activity

Bacteria	Presence of bubbles	Result (+/-)
<i>Staphylococcus aureus</i>		
<i>Streptococcus faecalis</i>		

H. Tryptophan Hydrolysis

Flick your inoculated culture to stir up the bacteria. Use a sterile swab to obtain some bacteria. Make sure you swirl the swab around in the broth so you pick up a large quantity of bacteria. Rub the swab on one area of the BBL Dry Slide. Record any color changes that occur between 30 – 60 seconds. **Do not wait too long before you observe your result**, as false positives can occur if results are not read right away.

Record your results in the table below.

Tryptophan Hydrolysis

Bacteria	Color	Result (+/-)
<i>Escherichia coli</i>		
<i>Enterobacter aerogenes</i>		

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7.4: Review Questions

1. Why do you think it is important to do spot-inoculations for the amylase test and the caseinase test?
2. What happens if you drop H₂O₂ into a cut on your arm? What does this tell you about the type of enzymes your cells might produce?
3. (a) You inoculate an organism into phenol red lactose broth. The organism ferments lactose.
What color is the medium? _____
What does this tell you about the pH of the medium? _____
(b) The organism ferments lactose and produces gases.
What do you use to detect gas production? _____?
What do you see that indicates that gases are produced? _____
4. In what test is Kovac's reagent used? _____
What molecule does Kovac's reagent detect? _____
To do this test, why do you have to grow the organism in medium containing tryptophan?
5. In the urea test, what happens to the pH of the medium when an organism breaks down urea? What molecule causes this pH change?
6. After inoculating a tube of nutrient gelatin, you incubate it overnight at 37° C. When you remove the tube from the incubator, you notice that the gelatin has liquefied. Does this indicate that the organism produces gelatinase? Why or why not?
7. Fill in the following table to summarize the metabolic tests that you learned about.

Summarize the metabolic tests

Test	Biochemical reaction/ Enzyme involved	Medium used/Inoculation Procedure	Appearance of Result: Positive Result	Appearance of Result: Negative Result	What causes the change in the medium's appearance? (e.g. pH change, enzyme activity, etc.)
Lactose Fermentation					
Gelatinase					
Amylase					
Urease					
Citrate Permease (Simmon's citrate test)					
Catalase					
Tryptophanase (Indole test)					

Results for Metabolic Tests:

Results

Bacteria	Gram stain	Morphology	Arrangement	Lactose Fermentation	Gelatinase	Amylase	Urease	Citrate permease (Simmons citrate)	Catalase	Tryptophanase (Indole test)
Bacillus subtilis										
Corynebacterium xerosis										
Enterobacter aerogenes										
Escherichia coli										
Micrococcus luteus										
Pseudomonas aeruginosa										
Proteus vulgaris										
Staphylococcus aureus										
Streptococcus faecalis										
Serratia marcescens										

The tests in the table above will be available for your practical exam. Use your results from Lab 5 to fill out this table. Results for the bacteria that were not tested in Lab 5 can be found on the Microbiology Review site on Blackboard.

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CHAPTER OVERVIEW

8: DNA extraction

[8.1: Background](#)

[8.2: Procedure and Review](#)

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8.1: Background

Background Information

Within the chromosomes of organisms, specific sections of DNA, called genes, code for specific proteins. A gene is “read” through a process of transcription to generate a sequence of mRNA. The mRNA is then used as the blueprint instructions to generate a specific protein during a process called translation. The process of generating a protein from a gene is often referred to as gene expression (Figure 1). A change, or mutation, in DNA can generate a protein that has a different amino acid sequence and therefore an altered or malfunctioning protein. Mutations in DNA of human chromosomes that cause malfunctioning or absent proteins are often the cause of genetic diseases. This lab discusses biotechnological advances that are used to detect and treat genetic diseases, advance agriculture or treat infectious diseases.



Figure 1. Gene expression. The process of transcription generates mRNA from a gene. mRNA undergoes translation to generate a protein. A change in the DNA sequence can alter the amino acid sequence of the protein and therefore can change or stop the function of the protein.

The first step in working with nucleic acids (DNA and RNA) is to remove the molecules from inside the cell. Different types of cells need to be processed differently in order to release nucleic acids. All cells have a **cell membrane**, a phospholipid bilayer that separates the internal environment of the cell from the external environment. In eukaryotes, DNA is housed inside the nucleus of the cell, which is surrounded by the **nuclear membrane**, a second double-layered membrane, also composed largely of lipid molecules. When extracting DNA from plant cells, the **cell wall** must also be considered; some types of plant tissue require grinding or flash-freezing in order to break the tough cell wall.

In the DNA extraction procedure, plant cell walls and cell membranes are broken down by blending or mashing the cells. There are two key ingredients in the DNA extraction buffer aside from the water: **dish soap (detergent)** and **salt**. The **detergent** in the extraction buffer solution acts to dissolve phospholipids that form the cell membrane and the nuclear membrane. When the phospholipid membranes are dissolved, the cell lyses and releases the cellular contents, including DNA. The **salt** has two functions in the extraction process. It helps to neutralize the charge on the sugar-phosphate backbone, making DNA *less soluble* in water and allowing it to more easily precipitate when the alcohol is added. The salt also helps to remove the proteins that are bound to the DNA and to keep the proteins dissolved in the lysis solution. The technique of filtration uses a medium, in this case, cheesecloth, to separate solids from liquids. The resultant material is referred to as filtrate. When cold **ethanol** is added to the filtrate, DNA precipitates at the water/ethanol interface. Although an individual DNA molecule is not visible with the naked eye, DNA isolated from large quantities of cells can be observed.

This protocol for the extraction of DNA is based largely on the principle of **solubility**. Solubility refers to the ability of one substance (the solute) to dissolve in another substance (the solvent). Polar substances dissolve easily in polar solvents, but do not dissolve easily in nonpolar solvents, a phenomenon commonly referred to as “like dissolves like.” Water is a polar solvent, and molecules that dissolve easily in water are referred to as **hydrophilic**. DNA molecules are hydrophilic because the sugar-phosphate backbone of the molecules is highly polar. This means that DNA dissolves in water, so in this experiment, the DNA that is released when the cells are crushed dissolves in the juice/extraction buffer mixture.

Although the chemical reactions described above are all happening when you add the buffer and crush the food (strawberries), they are not visible with the naked eye. However, the addition of the cold ethanol caused a much more dramatic result! Ethanol is a nonpolar solvent, and when it is added to the juice extract, the DNA precipitates out of the solution. A precipitation reaction is a chemical reaction that causes a solid substance to emerge from a liquid solution. In this experiment, the addition of ethanol to the reaction forces the DNA to precipitate out of solution, which we can then spool onto the wooden stirrer or glass rod.

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8.2: Procedure and Review

Exercise 1: DNA Extraction

Materials needed

1. Isopropyl alcohol 91% (rubbing alcohol) or 95% ethanol, Chilled in the freezer
2. Graduated cylinders
3. Salt
4. DI Water
5. Cheesecloth
6. Dishwashing liquid (preferably, Dawn)
7. Wide-mouth glass test tubes
8. Funnel
9. Disposable plastic cups
10. Microcentrifuge tubes
11. Wooden stirrer or glass rod
12. Resealable plastic bag
13. 100 or 200 mL beaker
14. Ice buckets with ice
15. Strawberries (a DNA source)

Preparation

- Have isopropyl alcohol (rubbing alcohol) or ethanol cooled in an ice bucket
- If not already made, prepare DNA extraction (lysis) buffer by combining the following:
 - 45 mL DI water
 - 5 mL liquid dish soap
 - 0.75 g NaCl (table salt)

Procedure

1. Place the a strawberry into the plastic zipper bag. Seal it and gently smash the strawberry with your hands for about 2 minutes. Completely crush it to disrupt the cells.
2. Open the plastic zipper bag and add 10 mL of the DNA extraction (lysis) buffer. Squeeze the bag to remove all air and seal the bag tightly.
3. Gently (to prevent over-sudsing or excessive foaming), but thoroughly, continue to crush the food (strawberries) inside the bag for about one minute or until it is a slushy consistency.
4. Completely line the funnel with a layer of cheesecloth. Place the funnel into the wide-mouth test tube.
5. Pour the food juice/DNA extraction buffer mixture into the funnel so the juice passes through the cheesecloth and into the test tube. Use the cheesecloth to strain the mixture so that only the juice flows into the tube and the pulp is retained in the cheesecloth.
6. Discard the cheesecloth and the pulp. Remove the funnel from the tube. The glass tube now contains a liquid called “filtrate”.
7. Carefully and slowly pour 10 mL of ice-cold ethanol on top of the filtrate in the test tube using the plastic transfer pipette. The alcohol is less dense than the filtrate and will float as a layer on top of the filtrate. Do not mix or stir!
8. Hold the tube still at eye level and observe what happens at the interface of the alcohol and the filtrate. DNA will precipitate at the alcohol-lysis buffer interface. Precipitate means it will come out of solution into a “solid” form and appear as fluffy white cotton or cloudy material. Verify with your instructor that you have isolated DNA.
9. (Optional) Use your wooden stirrer or glass rod to facilitate further precipitation.

Questions for Review

1. Explain the purpose of the different reagents:
 - Which reagent in the functions to dissolve membranes?

- Which reagent functions to remove proteins from DNA?
 - Which reagent precipitates DNA so that it can be seen with the naked eye?
2. Explain why the DNA dissolves in the lysis buffer and precipitates with the addition of alcohol. In your explanation, use your understanding how hydrophilic and hydrophobic components interact.
 3. What is the purpose of DNA in living organisms?
 4. How might changes in DNA affect an organism? Be specific and include the central dogma in your explanation.
 5. Provide two reasons scientists would need to extract DNA from cells (it can be any type of cell not just strawberries).
-

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CHAPTER OVERVIEW

9: Antimicrobials

9.1: Bacterial Susceptibility to Antibiotics (Kirby-Bauer Test)

9.2: Procedure

9.3: Results

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9.1: Bacterial Susceptibility to Antibiotics (Kirby-Bauer Test)

Learning Objectives

- Define "antibiotic" and tell where these chemicals come from in nature (for non-synthetic or non-semisynthetic).
- Differentiate between broad-spectrum antibiotics and narrow-spectrum antibiotics.
- Tell that use of broad-spectrum antibiotics contributes to increased antibiotic resistance in bacteria.
- Tell the purpose of the Kirby-Bauer test.
- Explain how the Kirby-Bauer test works, including how diffusion of the antibiotics is important in the creation zones of inhibition.
- Define "zone of inhibition."
- Explain why the Kirby-Bauer test is important for reducing antibiotic resistance.
- Describe why antibiotic resistance is a threat for successful treatment of bacterial infections.

Introduction to Antibiotics

Antibiotics are chemicals produced by some bacteria and fungi that, in small quantities, can inhibit the growth of bacteria. Much of the success we have achieved in treating infections since World War II is due to the discovery of antibiotics. Utilizing the information gleaned from studying these natural chemicals, scientists have artificially synthesized other useful antimicrobial chemicals in the laboratory. Sometimes these antimicrobials are completely synthesized in the lab (synthetic) and sometimes they are partly produced in nature and partly synthesized in the lab (semisynthetic).

Many microbes can produce antibiotics, but four genera produce most of the antibiotics used for treating human and animal infections. *Bacillus* and *Streptomyces* are bacteria. *Penicillium* and *Cephalosporium* are fungi. It is a constant challenge to develop new antibiotics to replace those antibiotics for which microbes have developed resistance.

The range of bacteria killed by an antibiotic determines its "spectrum of activity". Antibiotics that are only effective against Gram-positive or only effective against Gram-negative bacteria have a narrow spectrum of activity. Antibiotics that are effective against many different types of bacteria are called **broad-spectrum antibiotics**. Broad-spectrum antibiotics are probably contributing to the escalating drug resistance we are seeing in microorganisms. Broad-spectrum antibiotics often wipe out a person's normal microbiome as well as the pathogen they are intended to kill, resulting in superinfections from organisms such as *Candida albicans* and *Clostridium difficile* that grow out of control when they do not have to compete with microbes in the normal microbiomes.

Empiric therapy takes place when an antimicrobial agent is given to the patient without performing a culture or other diagnostic test to determine the specific cause of the disease. Empiric therapy is prescribed in instances where the causative pathogen is likely and where diagnostic tests will not change the treatment. The selection of which drug to use is based solely on experience, observation, and relevant clinical information, including current resistance patterns in suspected pathogens. These antibiotics are typically broad-spectrum, in that they treat a wide variety of possible microorganisms. Examples of this include antibiotics prescribed for strep throat, pneumonia, urinary tract infections, and suspected bacterial meningitis in newborns aged 0 to 6 months.

Physicians are beginning to target infections with narrow-spectrum antibiotics, or synergistically treat infections with small doses of multiple antibiotics to try to prevent antibiotic resistance.

The laboratory can aid the physician in selecting which antimicrobial agent is likely to kill the pathogen that is causing an infection in a patient. There are several methods used by clinical microbiologists in this determination, including the **Kirby-Bauer Test**.

Kirby-Bauer Test

The results of the Kirby-Bauer Test provide an accurate prediction of which antibiotics are likely to be effective against the pathogen. Because the Kirby-Bauer test is relatively simple to perform and is inexpensive, it has been extensively used in medical practice. The results are reported as **S (sensitive)**, **I (intermediate)**, and **R (resistant)** to an antibiotic. A sensitive result indicates the bacteria will die when it is exposed to the antibiotic. An intermediate result indicates the antibiotic must be used in combination with another antibiotic to clear the infection. A resistant result indicates the antibiotic does not kill the bacteria.

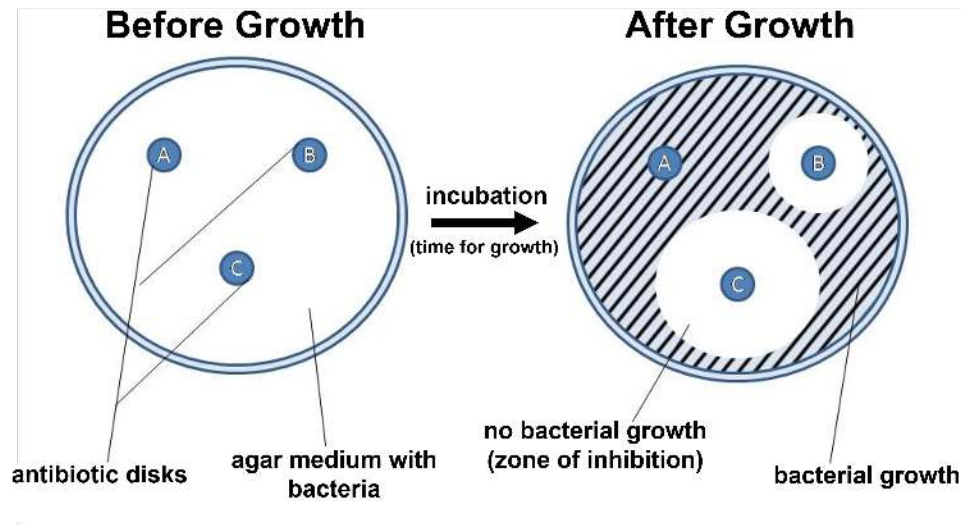


Figure 1: A summary diagram showing how the Kirby-Bauer approach works. A petri plate with nutrient agar is spread with the bacteria being tested. Antibiotic disks are placed on the agar. After incubation, zones of inhibition will occur surrounding the antibiotic disks based on the bacteria's sensitivity to the antibiotics tested. The zone of inhibition is a region where no bacterial growth occurred since it was inhibited/killed by the concentration of antibiotic present on the petri plate at that location (based on the diffusion of the antibiotic away from the antibiotic disk).

Petri plates with nutrient agar are covered with the bacteria that are being tested for antibiotic sensitivity. After spreading the bacteria over the entire surface of the petri plates, small disks containing different antibiotics are placed at a distance from each other (or alone) on the petri plate. Each antibiotic will diffuse outward from the antibiotic disk, creating a concentration gradient of the antibiotic in a circle surrounding the disk. Closest to the antibiotic disk will be the highest concentration of antibiotic. Further from the antibiotic disk will be the lowest concentration of antibiotic. Based on how sensitive the bacterial species/strain is to each antibiotic, bacteria will begin to grow at one of the antibiotic concentrations. Bacteria that can grow at higher concentrations of an antibiotic (closer to the disk) will have more resistance to that antibiotic. Bacteria that can only grow at lower concentrations of an antibiotic (further from the disk) will be more susceptible to that antibiotic. As a result, the size of the **zone of inhibition** (the region of the petri plate surrounding the antibiotic disk that does not have bacterial growth) will determine if the bacterial species/strain is sensitive, intermediate, or resistant to that antibiotic.

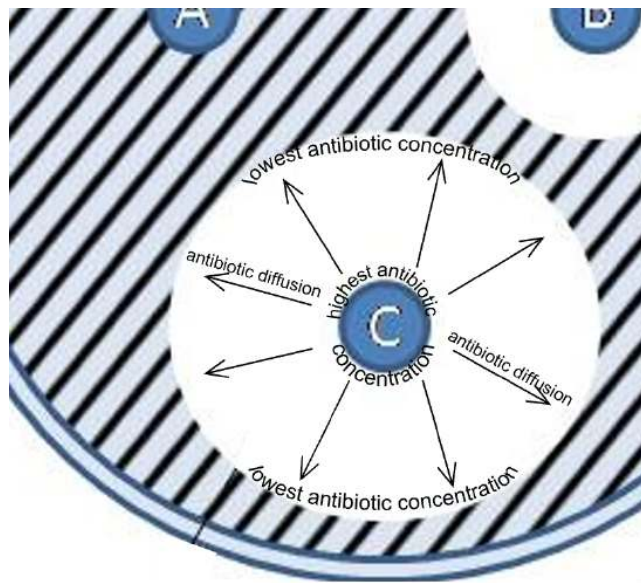


Figure 2: A closer look at antibiotic diffusion and how this produces zones of inhibition. Closest to the antibiotic disk is the highest antibiotic concentration and the antibiotic diffuses outward to produce a concentration gradient of antibiotic. The further from the antibiotic disk, the lower the antibiotic concentration. This approach enables a quick way of seeing a spectrum of antibiotic concentrations and how this impacts inhibition of the bacterial species/strain being tested.

The Kirby-Bauer test, known as agar disk diffusion, must be strictly regulated for the results to be interpreted correctly. Such characteristics as the stability of the antibiotic, the rate of diffusion of the antibiotic, the bacteria being tested, the pH of the culture medium, the depth of the culture medium, the inoculum density, the incubation time, the incubation temperature, and the concentration of the antibiotic can affect the results. Nevertheless, when the Kirby-Bauer test is performed under standardized conditions (on Mueller-Hinton agar inoculated with a pure culture of microbes that is the correct turbidity to match McFarland 0.5 standard, etc.) and the results are interpreted according to the Interpretative Zone Standards published by the National Committee for Clinical Laboratory Standards. In this laboratory, this is a simplified version of the Kirby-Bauer Test in the lab that is not standardized, but will allow you to learn the general principles involved in this procedure.

Attributions

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9.2: Procedure

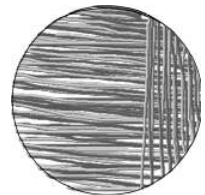
MATERIALS NEEDED: per table

- 2 Mueller-Hinton agar plates
- 24 hr old cultures (likely to be Staph, E. coli, Bacillus subtilis, Enterococcus faecalis) sterile swabs
- antibiotics
- ethanol
- forceps
- *Pseudomonas aeruginosa* Kirby-Bauer plate for demo
- **guidelines chart** for interpretation of antibiotic susceptibility

THE PROCEDURES

1. Swab a Mueller-Hinton plate with **ONLY 2** of the bacteria (tables will run different combinations of the 4 bacteria). Dip a sterile swab into the broth and express any excess moisture by pressing the swab against the side of the tube.
2. Swab the surface of the agar completely (you do not want to leave any unswabbed agar areas at all). In the pictures on this page, you can see what happens when the plate is not swabbed correctly with even coverage of the bacterium over the entire agar.
3. After completely swabbing the plate, turn it 90 degrees and repeat the swabbing process. (It is not necessary to re-moisten the swab.) Run the swab around the circumference of the plate before discarding it in the discard bag.
4. Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar.
5. THE ANTIBIOTIC DISKS:
 - You are using individual antibiotic dispensers.
 - You will probably have to use a pair of forceps to remove an antibiotic disc from the dispenser: the forceps must be sterile. Place the forceps in alcohol, flame the forceps until they catch on fire, let the flame go out--**sterile forceps**.
 - Lightly touch each disc with your sterile inoculating loop to make sure that it is in **good contact with the agar surface**. Incubate upside down and incubate at 37° C.

2nd swab coverage ►



INTERPRETATION

1. Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge. **HOLDING THE PLATE UP TO THE LIGHT MIGHT HELP.**
2. Use millimeter measurements. The disc diameter will actually be part of that number.
3. If there is NO zone at all, report it as 0---even though the disc itself is around 7 mm.
4. Zone diameter is reported in millimeters, looked up on the **chart**, and result reported as sensitive, resistant, or intermediate.
5. Record the results for your table, as well as other tables, in the table.



Table 9.2.1: GUIDELINES CHART

Antibiotic (Antimicrobial Agent)	DISC CODE	Resistant (< or = mm)	Intermediate (mm)	Susceptible (= or > mm)
Amoxicillin (other)	AMC	<13	14-17	>>18
Amoxicillin (Staph)	AMC	19		>20
Ampicillin (other)	AM	11	12-13	>14
Ampicillin (Staph)	AM	28		>29
Carbenicillin (other)	CB	17	18-22	>23
Carbenicillin (Pseudomonas)	CB	13	14-16	>17
Cefoxitin	FOX	14	15-17	>18
Cephalothin	CF	14	15-17	>18
Chloramphenicol	C	12	13-17	>18
Ciprofloxacin	CIP-5	15	16-20	>21
Clindamycin	CC-2	14	15-20	>21
Enoxacin (Fluoroquinolone, 2nd gen.)	ENX-10	14	15-17	>18
Erythromycin	E	13	14-22	>23
Gentamycin	GM	12	13-14	>15
Kanamycin	K-30	13	14-17	>18
Methicillin (Staph)	M(orDP)	9	10-13	>14
Oxacillin (Staph)	OX	10	11-12	>13
Penicillin G (Enterococcus)	P	14		>15
Penicillin G (Staph)	P	28		>29
Streptomycin	S-10	14	15-20	>21
Sulfamethoxazole-trimethoprim	SXT	10	11-15	>16
Tetracycline	Te-30	14	15-18	>19
Tobramycin	NN-10	12	13-14	>15
Vancomycin	Va-30	9	10-11	>12

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9.3: Results

Determine if each strain is resistant, intermediate, or susceptible to each antibiotic

	<i>S. aureus</i>		<i>E. coli</i>		<i>B. subtilus</i>		<i>E. fecalis</i>		<i>P. aeruginosa</i>	
Antibiotic	Zone (mm)	S, R, or I?	Zone (mm)	S, R, or I?	Zone (mm)	S, R, or I?	Zone (mm)	S, R, or I?	Zone (mm)	S, R, or I?

1. Complete the table above using measurements of the diameter of the zones of inhibition and the interpretation table to determine if each strain is resistant, intermediate, or susceptible to each antibiotic.
2. If you had a patient with an infection of this strain of *S. aureus*, which antibiotic(s) might be a good choice for treatment? Explain your answer.
3. Define "zone of inhibition."
4. Explain how the size of the zone of inhibition relates to the concentration of the antibiotic.
5. Explain how the size of the zone of inhibition relates to the susceptibility or resistance to an antibiotic.
6. What is "antibiotic resistance?"
7. Why is using this Kirby-Bauer approach helpful to prevent bacterial strains from becoming resistant to antibiotics?
8. **True or False.** It is easy for scientists to develop new antibiotics.
9. What are the treatment options for a bacterial infection where the bacterial species is resistant to all types of antibiotics?
10. Why is it important to take steps to prevent bacterial strains from becoming antibiotic-resistant?

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CHAPTER OVERVIEW

10: Microbiology of Milk and Food

[10.1: Introduction](#)

[10.2: Procedures](#)

[10.3: Results](#)

[10.4: Review Questions](#)

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10.1: Introduction

Learning Objectives

- Learn how to use serial dilutions.
- Learn how to do a standard plate count.
- Understand the importance of standard microbiological tests for food safety

Pathogens can be introduced into foods at any stage: during growth/production at the farm, during processing (grinding, chopping, milling, etc.), during handling and packaging, and when the food is prepared in the kitchen. In many cases, small numbers of pathogenic bacteria are not dangerous, but improper storage and/or cooking conditions can allow these bacteria to multiply to dangerous levels.

Fecal contamination of water (and through water, contamination of food materials) is another way in which pathogens can be introduced. **Coliform bacteria** are gram-negative, non-spore-forming bacteria that are capable of fermenting lactose to produce acid and gas. A subset of these bacteria is the **fecal coliforms**, which are found at high levels in human and animal intestines. Fecal coliform bacteria, such as *E. coli*, are often used as **indicator species**, as they are not commonly found growing in nature in the absence of fecal contamination. The presence of *E. coli* suggests feces are present, indicating that serious pathogens, such as *Salmonella* species and *Campylobacter* species, could also be present.

In this lab, you will examine bacteria found in milk, chicken, and in other assorted food materials.

Milk

Milk contains carbohydrates, minerals, fats, vitamins, and proteins, and is therefore susceptible to breakdown by a wide variety of microorganisms. Several different kinds of bacteria may be present in milk, most commonly the genera *Lactobacillus*, *Micrococcus*, and *Streptococcus*. As discussed in Lab 7, regular pasteurization is the process used to reduce microbial loads to acceptable levels in foods like milk and fruit juices. Milk that has undergone a regular pasteurization procedure can still contain bacteria. If this milk is stored at 4°C (refrigerator temperature) the bacteria are prevented from multiplying, but if the milk is left out at room temperature, the bacteria will reproduce, and the milk will spoil.

If done aseptically, **UHT (ultra-high temperature) pasteurization** can sterilize foods. This is why UHT milk can be left unopened at room temperature for long periods of time.

Microorganisms of concern in milk include *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, *Salmonella* species (most common: *S. enteritidis* and *S. typhimurium*), and *Yersinia enterocolitica*.

Chicken

Raw poultry products are frequently contaminated by pathogens (e.g. *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter jejuni*, etc.). Salmonellosis is one of the most common serious foodborne infections caused by contaminated poultry. The prevalence of *Salmonella* in raw fresh and frozen poultry approaches 80% in some countries.

Fruits and Vegetables

Fruits and vegetables may become contaminated with potential pathogens from the soil or water they come in contact with while growing. They may also pick up harmful bacteria during processing, storage, and preparation. While the use of potable (uncontaminated, drinkable) water to wash and fresh, harvested fruits and vegetables is useful both commercially and in the home, residual water may support extensive microbial growth during further storage.

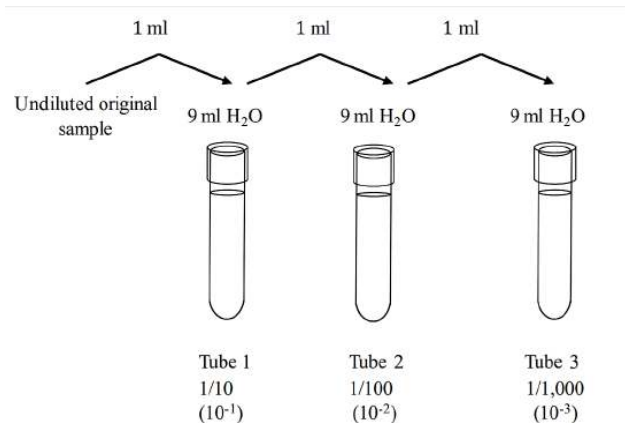
Standard Plate Count and Serial Dilution Techniques

One of the most common methods of determining the amount of bacteria in a food product is the **standard plate count**. In this method, serial dilutions of the food are plated on general-purpose and/or differential/selective growth media. Bacterial colonies are

then counted, and the number of **colony-forming units (CFUs)** in the original undiluted sample is calculated. Note: CFUs are a measure of the number of bacteria to take into account that one colony might be the product of more than a single bacterium.

Serial dilution is a technique that is used to produce very dilute solutions without the necessity of measuring very small quantities of liquids. It is a series of stepwise dilutions, in which one first dilutes a solution, then dilutes the dilution, then dilutes the dilution of the dilution, and so forth. The dilution factor at each step is usually constant, resulting in a geometric progression of concentration. An example of a serial dilution is seen below. In this example, each dilution is a 10-fold dilution (transferring 1 ml into 9 ml of H₂O results in a 1/10 dilution, i.e., 1 ml in a total volume of 10 ml).

Serial dilutions are often used in standard plate counts because the number of bacteria in a sample (water, food, or a medical sample such as a urine or fecal sample) is unknown. The sample is diluted to obtain several CFUs that supply statistically significant results, yet it is still easily countable. The general recommendation for a countable plate is between 30 – 300 CFUs/plate.



F
figure
10.1.1:
Serial Dilution

After dilutions are prepared, a set amount of liquid (typically between 0.1- 1 ml) is spread out over the surface of an agar plate, and then incubated to allow for bacterial growth. CFU counts from these diluted plates are used to calculate the number of bacterial cells/ml in your original (undiluted) sample. If you plate a full milliliter (ml) of your dilution, you would simply multiply the number of CFUs counted by the dilution factor of the plate you counted.

- Example: I count 55 CFUs on a plate diluted 1:1000 (10^{-3}) in which I added a full ml of my dilution. My calculation is $55 \times 1000 = 55,000$ (5.5×10^4) cells/ml.

However, it is important to note that we do not always plate a full ml (it can be difficult to get that much liquid to be absorbed into an agar plate). Therefore, an additional calculation is often necessary to be able to express your results as cells/ml. If you plate 0.1 ml of sample, you will need to multiply the number of CFUs by 10 to determine # CFUs/ml. If you plate 0.5 ml of a sample, you will need to multiply by 2 to determine the number of CFUs/ml.

- Here's another example: you set up serial dilutions for a milk sample and plate out 0.5 ml of your dilutions. The countable plate that came from your 1:100 (10^{-2}) dilution has 114 colonies.

Your calculation:

1. Multiply: #CFU's x dilution factor = $114 \times 100 = 11400$ cells/0.5 ml.
2. Multiply by 2 to express your result as cells/ml $11400 \times 2 = 22800$ cells/ml (or 2.28×10^4) If dilutions are done properly, we should expect to see a geometric progression in the number of cells in each dilution as shown below.

Results of multiple dilutions

	Dilution	Number of bacteria/ml in each tube after dilution	Number of CFUs (1 ml plated)	Number of CFUs (0.1 ml plated)
Undiluted sample		1,000,000/ml = 10^6 /ml	1,000,000 (10^6)	100,000 (10^5)
Tube 1	$1/10 = 10^{-1}$	100,000/ml = 10^5 /ml	100,000 (10^5)	10,000 (10^4)
Tube 2	$1/100 = 10^{-2}$	10,000/ml = 10^4 /ml	10,000 (10^4)	1,000 (10^3)
Tube 3	$1/1000 = 10^{-3}$	1,000/ml = 10^3 /ml	1,000 (10^3)	100 (10^2)

Your results would not turn out as perfectly as those shown above. Slight inaccuracies in pipetting would cause variations in the final results. In the real world, the results of multiple dilutions are averaged together when determining the final number of bacteria/ml in a sample.

For a review of scientific notation and additional practice problems to help you understand serial dilution, see Appendix II. For additional information about the use of pipettors for measuring small volumes, see Appendix III.

Key Terms

CFU, indicator bacteria, serial dilution, coliform bacteria, fecal coliform bacteria, standard plate count, UHT pasteurization

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10.2: Procedures

A. The Microbiology of Milk

In this exercise, you will compare the number of bacteria found in regular pasteurized milk with the amount found in UHT pasteurized milk. You will also examine the effects of leaving the two different types of milk at room temperature.

Per pair of students: 2 TSA plates

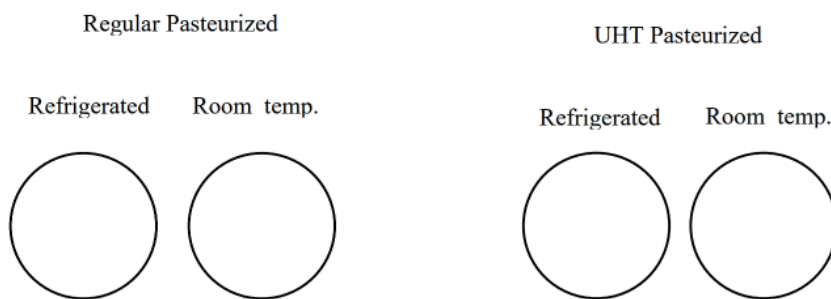
Per pair of students:

Either: 2 tubes of pasteurized milk: one refrigerated, one left out at room temperature for 1 week, **or** 2 tubes of UHT pasteurized milk: one refrigerated, one left out at room temperature for 1 week

1. Each pair of students will choose either the regular pasteurized milk or the UHT pasteurized milk.
2. Using the 20-200 μl pipettor (also called the P200), transfer 200 μl of the refrigerated milk on a TSA plate. (Appendix III provides instructions on how to use micropipettors.)
3. Use a sterile spreader to spread the 200 μl of the milk across the plate.
4. Sterilizing the spreader between each plate, repeat Steps 2-3 using the room temperature milk.
5. Incubate the plates until the next lab.

Type of milk chosen: _____

Per Table, you should have the following plates:



What type of bacteria will grow on the TSA plates? _____

Predict which type of milk will have the most bacteria. Explain the rationale for your prediction.

B. The Microbiology of Poultry

In this exercise you will determine the number of bacteria found on chicken wings using serial dilutions followed by a standard plate count.

There will be 1 resealable plastic bag containing 9 ml of sterile water and a chicken wing at the instructor's table.

Per table: 3 TSA plates ,4 tubes, each containing 9 ml of sterile H_2O , 1 tube of chicken wing wash fluid (given to you by your instructor)

1. The instructor will rinse the chicken wing in the water, massaging the chicken wing to remove any bacteria that are adhering to the skin. Using a 200 - 1000 μl pipettor (also called the P1000), the instructor will transfer 1 ml of the wash fluid into 99 ml of H_2O and will mix thoroughly. This will be a dilution of 1/100 (10^{-2}). The instructor will transfer 2-3 ml of this 1/100 dilution into 4 sterile tubes (1/table).
2. Label one of the tubes of sterile water 10^{-3} (1/1,000)—this will be Tube 1. Label one of the tubes of sterile water 10^{-4} (1/10,000)—this will be Tube 2. Label the next tube of sterile water 10^{-5} (1/100,000)—this will be Tube 3. Label the last tube of sterile water

10^{-6} (1/1,000,000)—this will be Tube 4.

3. Using the P1000, remove 1 ml of the 1/100 wash fluid from the tube given to you by the instructor, and transfer it to Tube 1. Mix the contents of the tube by carefully flicking the tube. Using the same pipettor, transfer 1 ml from Tube 1 into Tube 2; transfer 1 ml from Tube 2 to Tube 3, and transfer 1 ml from Tube 3 to Tube 4. After each transfer, flick to mix thoroughly. The figure below shows you how to make the transfers.

Instructions for plating

Using the P200, transfer 100 μ l from Tube 2, Tube 3 and Tube 4 to 3 different TSA plates. Make sure to label your plates. The figure below indicates how the transfers should be done.

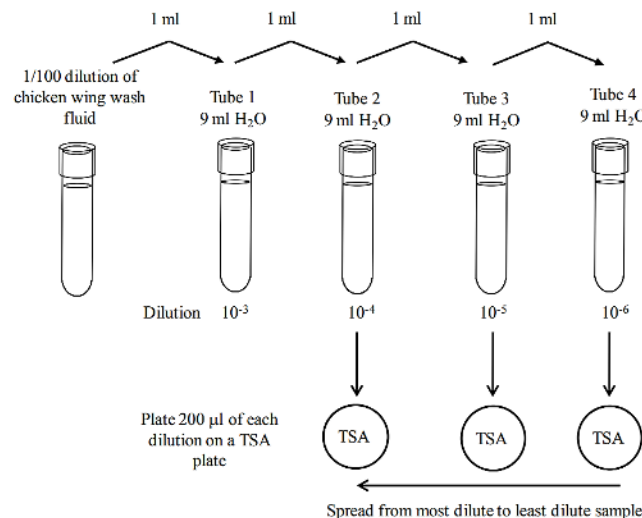


Figure 10.2.1: Serial dilutions and transfers to plates

You will be using 1 sterile spreader for all of your plates, so you must be careful to work from the most dilute sample to the least dilute sample. Make sure that all of the fluid has been absorbed into the plate before you invert the plate for incubation.

C. Microbiology of Food

Per table: 1 TSA plate

Per table: Choose 1 of the following four food materials: ground beef, mushroom, bread or lettuce.

Chosen food material: _____

1. Flame-sterilize forceps. (Note: follow the recommendations for forceps sterilization described in Lab 9.)
2. Pick up a portion of your chosen food product, gently rub it over the surface of the TSA plate, then discard the food.
3. Incubate the plate until the next lab. Of the four food materials, which do you think will contain the most bacteria?

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10.3: Results

A. The Microbiology of Milk

Record the results of your experiment in the table below. Record growth as 0, +, ++, or +++. Describe any notable growth characteristics.

TSA Plates

	Amount of Growth	Growth Characteristics
Regular pasteurized (refrigerated)		
Regular pasteurized (room temperature)		
UHT pasteurized (refrigerated)		
UHT pasteurized (room temperature)		

Which type of milk had the largest number of bacteria? _____

Was your earlier prediction correct? _____

B. The Microbiology of Poultry

Count the CFUs on your plates. Record your numbers in the table below. If there are too many CFUs to count, just write “TMTC” (too many to count). Remember—a countable plate that is considered statistically significant should have between 30 -300 CFUs.

Record your plate counts below. Make note of any notable growth characteristics (e.g., fungal-like growth, colony colors, etc.) Note: not all of your plates may be in the countable plate range (they may have many more or many fewer colonies). You need only record the numbers for the countable plates.

Based on the results for each countable dilution, determine the total number of bacteria in the original sample. Then average these numbers to obtain a more significant result.

Determine the total number of bacteria in the original sample:

Dilution plated	Amount spread on plate	Growth characteristics	Number of CFUs/plate	Number of bacteria/ml in original sample (No. CFUs x Dilution factor x 10)
Tube 2 = 10^{-4} 0.1 ml				
Tube 3 = 10^{-5} 0.1 ml				
Tube 4 = 10^{-6} 0.1 ml				

Average number of bacteria/ml in original sample:

If you also did this experiment using EMB plates, would you expect more colonies on the TSA or the EMB plates? Explain your answer.

What type of bacteria could you identify by using the EMB plates, and why would these bacteria be of concern for human health?

Class Data

For the results of the experiment to be more statistically significant, data from the entire class will be collected and written in the following table.

Data collected from each table

	Number of bacteria/ml in original sample
Table 1	
Table 2	
Table 3	
Table 4	
Average	

C. Microbiology of Food

Observe the plates of all of the food materials. Rate the food material based on the relative number of bacterial colonies seen on each plate:

least bacteria _____ < _____ < _____ < _____ most
bacteria

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10.4: Review Questions

1. You are testing ground beef to determine how many bacteria are present per gram of meat. You place 1 gram of meat in sterile water, mix vigorously, and then do serial dilutions as shown below. You inoculate a plate with 0.1 ml of the sample from the last tube. The next day you count 122 colonies. How many bacteria/ml were in the undiluted sample?

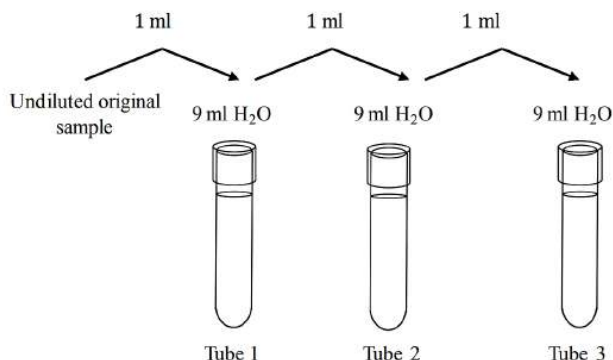


Figure 10.4.1: Serial Dilutions for Question 1

2. When doing serial dilutions, why is it necessary to plate more than one dilution?
3. If you observe *Escherichia coli* in a food sample, what could this indicate?
4. You left a carton of orange juice on your counter for 3 days. When you taste it, it is very bubbly (as though it were carbonated), and it tastes more bitter than usual. Note: Acids taste bitter. What do you think could have happened? (Note: you can't just say that bacteria grew—you must explain how the growth of the bacteria resulted in the changes in the orange juice.)
5. Based on what you now know about the presence of bacteria on chicken and beef, why is it a good idea to use separate chopping boards for meat and for vegetables?
6. You are testing unpasteurized milk for the presence of bacterial contamination. Starting from the undiluted milk, you do serial dilutions as shown below, and plate 1.0 ml of each dilution on agar. If the undiluted milk contains 5×10^6 bacteria/ml, how many colonies would you expect to see on each plate?

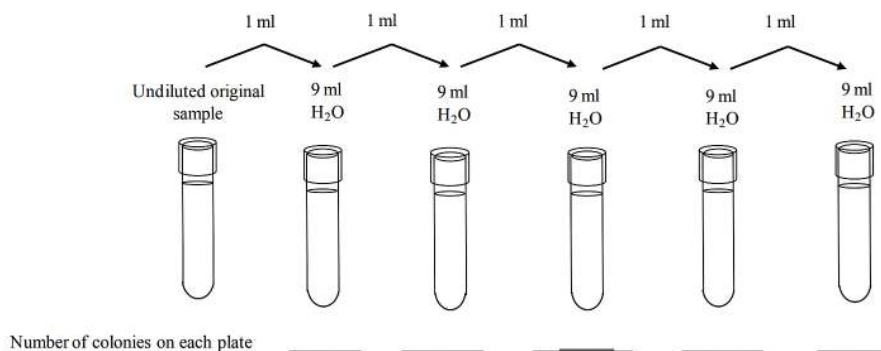


Figure 10.4.2: Serial Dilutions for Question 6

7. Do you think standard plate counts are very accurate? Why or why not?

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CHAPTER OVERVIEW

11: Epidemiology

11.1: Epidemiology and Public Health

11.2: Solve the Outbreak

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11.1: Epidemiology and Public Health

Epidemiology is a science that studies the causes and effects of health-related events as they occur in populations. Disease, defined as a deviation from health, is one such health-related event of concern to epidemiologists, so in that regard, epidemiology is often thought of as the study of disease in populations.

Although the historical origins of epidemiology as a science are investigations of epidemics of infectious disease, modern epidemiology has expanded to include not only contagious diseases, but also environmental connections to disease states and even accidental injuries. Epidemiologists gather data on the frequency of various diseases in populations and correlate risk factors associated with disease development.

The information compiled by epidemiologists provides the foundation for the concept of “public health.” The focus of public health is to prevent and manage diseases, injuries, and other conditions that threaten human health. Keeping track of the number of people who acquire or have a particular health-related condition guides the deployment of interventions, distribution of grant funding for research on particular diseases, and development of public health policy.

In the United States, the Centers for Disease Control and Prevention (CDC) is the arm of the federal government responsible for promoting and protecting public health. On the infectious disease front, the CDC receives reports on the occurrence of certain infectious diseases, called **notifiable diseases**, from regions in the United States and its territories. The data received from state and local health agencies each week is compiled into a large searchable database called the National Notifiable Diseases Surveillance System (NNDSS) and published in the *Morbidity and Mortality Weekly Report* (MMWR), which is available in both print and electronic formats.

The data maintained within the NNDSS tables is available for retrospective analysis and used to predict trends in disease occurrence in populations by time and place.

Two important measurements of disease occurrence and distribution are **morbidity** (illnesses due to a disease) and **mortality** (deaths due to a disease). The morbidity of a specific disease is defined as the number of susceptible people in a population who have the disease during a specific period and is usually expressed as a rate. Mortality may also be expressed as a rate and reflects the number of deaths due to a particular disease in a population over time.

Frequency of Disease in a Population

The frequency at which a disease occurs in a population is a way to assess risk and disease impact. One way to measure disease frequency is to simply count how many people are afflicted with it in a given period. However, using simple counts prevents comparison among populations, which may vary vastly in size. Therefore, disease frequency is usually expressed as a proportion of the number of people affected by the disease to the population size, over a specified time.

Two specific statistical measures widely used in epidemiological investigations are **incidence** and **prevalence**. Incidence is a measure of the number of NEW cases of a disease during a specific time period. Incidence is used as a way to understand risk factors, such as the cause of a health-related event or concern for disease spread. Prevalence refers to the total number of both new and existing cases in a population over time and indicates the overall health of the population during a time.

Both statistics measure the disease over time. For this reason, they are often expressed as a rate:

Incidence rate = Number of new cases of a disease in a population ÷ Number of at-risk people during a time period

Prevalence rate = Number of cases of a disease in a population ÷ Number of at-risk people during a time period

Because the number of cases of any disease may be small, and the size of the population under study may be very large, the resulting number may be so exceptionally small that it is perceived to be of no consequence. Therefore, these measures are often expressed as a percent, or multiplied by a factor of 100, 1,000, or even 100,000 so that the rates are expressed in number of people per 100, 1,000, or 100,000 individuals, respectively. For example, if over the course of one year, five women in a study population of 200 women (5/200) develop breast cancer, then the calculated incidence of breast cancer in this population is 0.025. Such a

small number might lead some people to presume their disease risk is also small. Therefore, the incidence may be expressed as a percent (2.5%), or a multiplier can be used to express the disease rate as 25 breast cancer cases per 1,000 women per year.

Prevalence estimates the likelihood that someone in a group will have a disease and is often used as an indicator of the overall healthcare burden of a disease. Prevalence is highly dependent on the duration of the morbidity associated with the disease. The prevalence of chronic diseases will continually increase as the cases accumulate over time, since it is a measure of both new and existing cases.

For example, a survey asking about personal experience with colon cancer was provided to 80,000 people, with 2,400 responding that they had been recently diagnosed with the disease, and 7,000 people responding that they'd had the disease for more than a year. The prevalence rate for colon cancer in this population can be determined by adding new and existing cases (9,400) and dividing by the size of the population (80,000). Therefore, the prevalence of cancer in this population is 0.1175, which can be expressed as 11.75%, or 118 colon cancer cases per 1,000 people.

Incidence and prevalence are two fundamentally different statistics. Keeping track of new cases of a disease requires an extensive network of reporting, while prevalence can be determined by surveying members of a population at a given point in time. Although there are limitations, if the disease is fairly stable in the population, has an average time of duration, and is not irreversible, incidence can be estimated using the prevalence data, and vice versa, using the following relationships (where Time refers to the average amount of time a person is sick with the disease):

$$\text{Prevalence rate} = \text{Incidence} \times \text{duration (in days, weeks, or months) of the disease}$$

$$\text{Incidence rate} = \text{Prevalence} / \text{duration (in days, weeks, or months) of the disease}$$

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11.2: Solve the Outbreak

Directions

Note: You will need to bring a laptop or tablet to class.

1. Navigate to the following website: <https://www.cdc.gov/mobile/applications/sto/web-app.html>
 2. With your partner(s) choose different cases to work on. For your case, complete a Case Report (see below) in your lab notebook.
 3. Report your findings and the outcome to your partner(s) and discuss what you found during the case studies. In four or more sentences, compare and contrast the different outbreaks in your case report.
 4. Repeat steps two and three until you have individually reported on **two** different cases.
 5. Answer the discussion questions at the end of the lab.
-

Case Report

Title of case:

Notes:

As you go throughout your case, take notes on the following:

- Who? List those who have been infected and/or exposed (e.g. how many people) and what information you have about them.
- What? List what are the symptoms of the infections and explain what the possible source or cause of the infection may be.
- Where? List where the infections have been reported and what other locations may be involved in the outbreak.
- When and how? List the timings of different infections and speculate on how the outbreak is spreading.

Summary

Summarize what you learned. Put all the information together in a succinct report of the evidence you gathered and how you used it to determine what happened.

Comparison report

Give a verbal report to your partner and exchange information with them about their cases. In four or more sentences, compare and contrast the pathogens, patients, and incidence patterns between one of your cases and one of your partner(s) cases.

Repeat the comparison report for the other case you and your partner(s) completed.

Discussion Questions

1. What is the study of epidemiology, and how is it connected to public health?
 2. What is the difference between an outbreak and an epidemic?
 3. How does the mechanism of disease transmission affect an outbreak? Include in your answer an example of two different diseases that may be prevented with different methods.
 4. How can addressing outbreaks when they happen prevent epidemics?
-

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CHAPTER OVERVIEW

12: Immunology

[12.1: Identification of White Blood Cells](#)

[12.2: Procedure and Results](#)

[12.3: Review Questions](#)

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12.1: Identification of White Blood Cells

Introduction

Today we will be exploring the white blood cells that are so important to the innate and adaptive immune system. All of your red and white blood cells originate from a type of stem cell called a hematopoietic stem cell. These cells are found primarily in your bone marrow, but also in your peripheral blood, and throughout your life, they differentiate into any of the red or white blood cells that are needed. Red blood cells have a short life span of about 120 days, so they are constantly being replaced. Many of your white blood cells have even shorter life spans, some of only minutes or days!

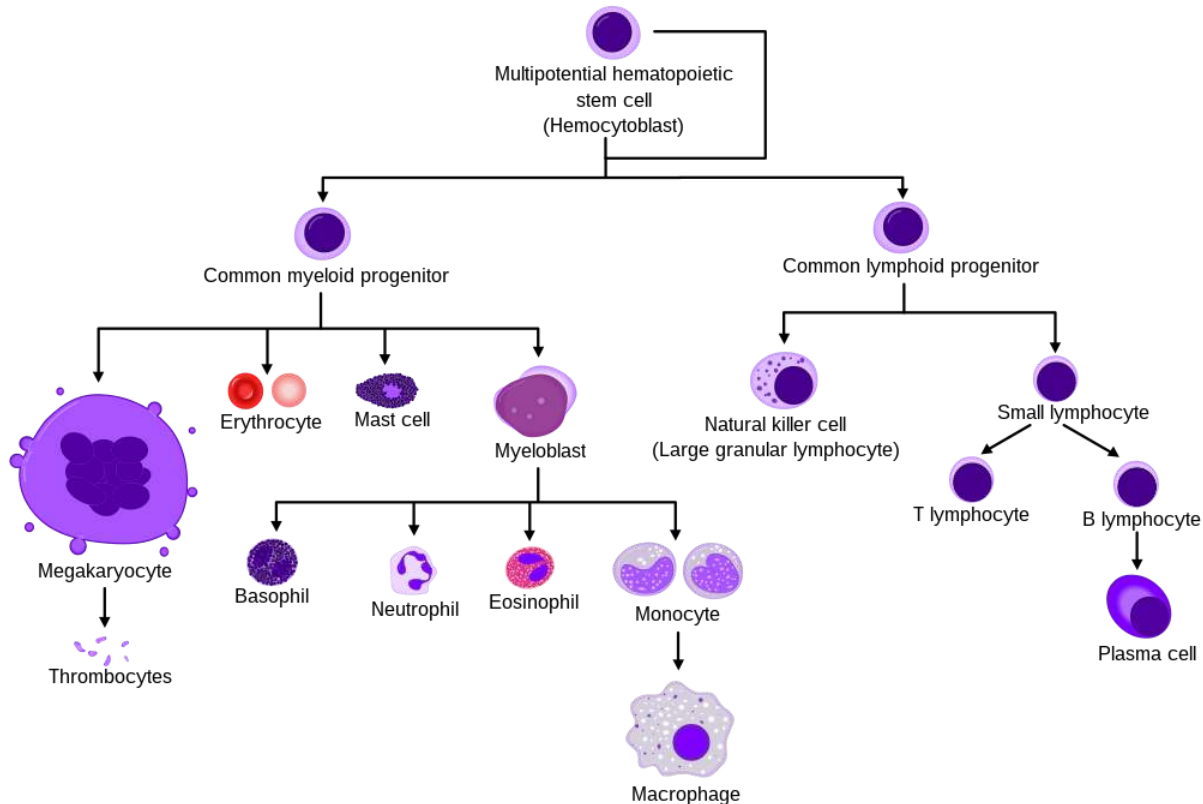
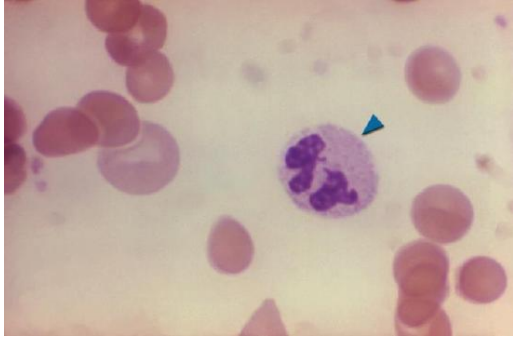
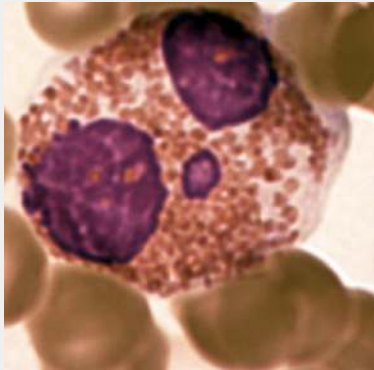
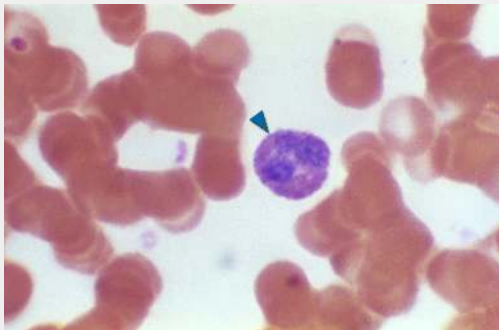
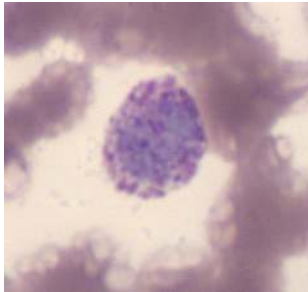
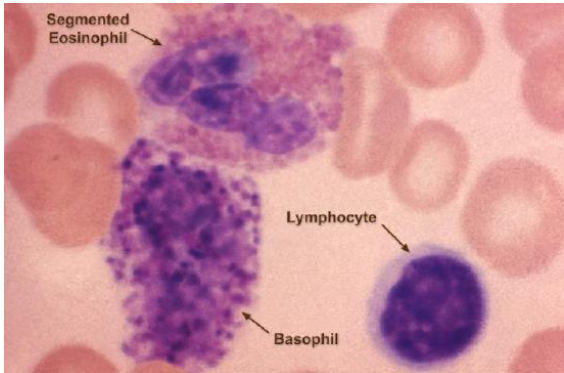
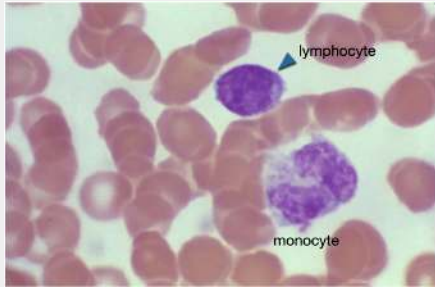
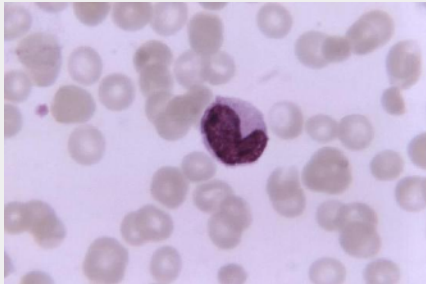


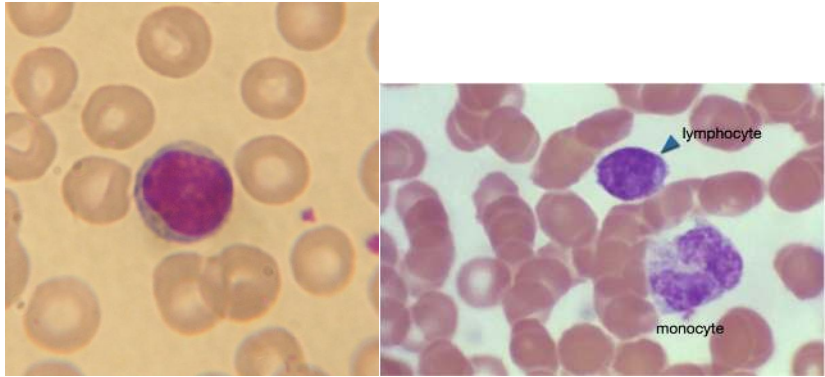
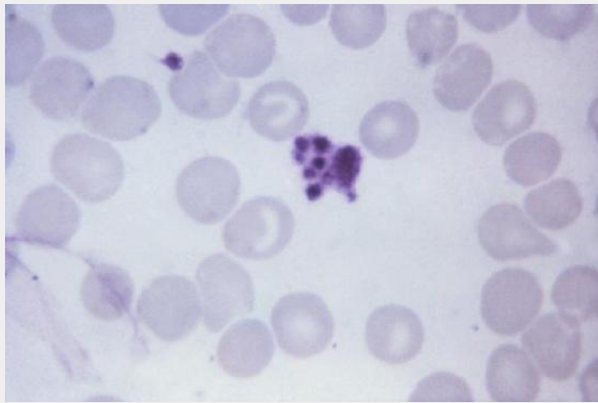
Figure 12.1.1: Simplified hematopoiesis. By A. Rad and M. Häggström, CC BY-SA 3.0, via Wikimedia Commons

In this Openstax textbook, [Chapter 17.3](#) describes the functions of each of these cells in detail, so these functions are not recapitulated here. However, you will need to be able to recognize them under the microscope. The table below will help you determine which cells are which.

Table 1: Examples of WBC's Seen in Peripheral Blood

Cell type	% found in blood	Images
Neutrophil	55-65%	  User CS99 at German Wikipedia, Public domain, via Wikimedia Commons (left image), CDC Public Health Image Library, Public Domain (right image).
Eosinophil	1-2%	  Davidcsaba Dr. David Csaba L., Public domain, via Wikimedia Commons (left), CDC Public Health Image Library, Public Domain (right image).

Cell type	% found in blood	Images
Basophil	0.2%	  CDC Public Health Image Library, Public Domain (right image).
Monocytes	3-7%	  CDC Public Health Image Library (left), and CDC Public Health Image Library (right) Public Domain

Cell type	% found in blood	Images
lymphocytes	25-33%	 Bernanke's Crossbow, CC BY-SA 3.0, via Wikimedia Commons (left image), CDC Public Health Image Library, Public Domain (right)
platelet	N/A	 CDC Public Health Image Library, Public Domain

Wright's Stain

Wright's stain contains the dyes eosin Y and methylene blue. These two stains bind differently to different white blood cell types and allow them to be easily differentiated from one another. Eosin Y stains the cytoplasm of cells a pink or orange color, while methylene blue stains the cellular nuclei a blue color. This makes it fairly straightforward to differentiate the various white blood cells from each other. Additionally, the granules of basophils and eosinophils are stained blue or red, respectively.

We will be using a pre-prepared blood smears with Wright's stain in today's lab.

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12.2: Procedure and Results

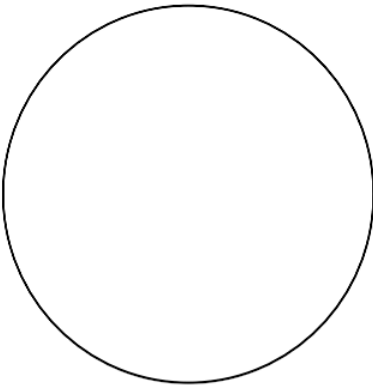
Procedure

1. Using both a low (4x or 10x) and high (40x) power objective lens, sketch one of each cell type.
2. Starting with the 10X objective, scan the prepared slide and count at least 50 **white** blood cells. Do not count platelets of red blood cells. As you count, record the numbers of each cell type in your data table.
3. Calculate the percentage of neutrophils, lymphocytes, monocytes, eosinophils, and basophils out of the total.

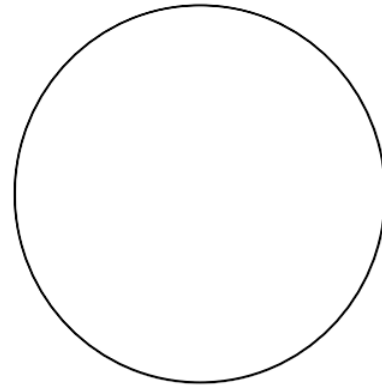
Results

Neutrophil

Low power



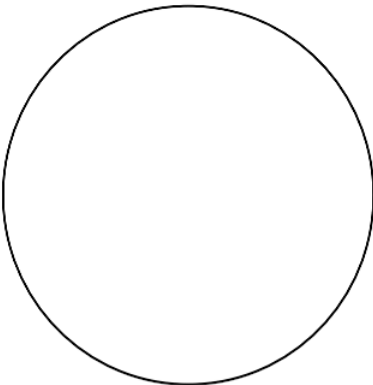
High power



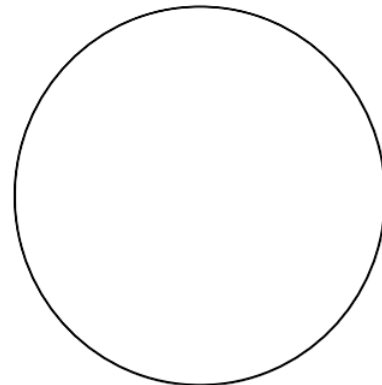
Total Magnification

Eosinophil

Low power



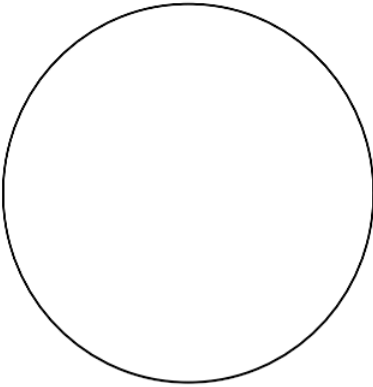
High power



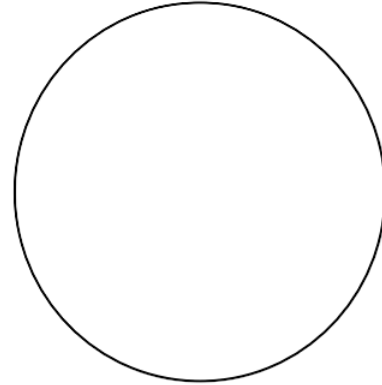
Total Magnification

Basophil

Low power



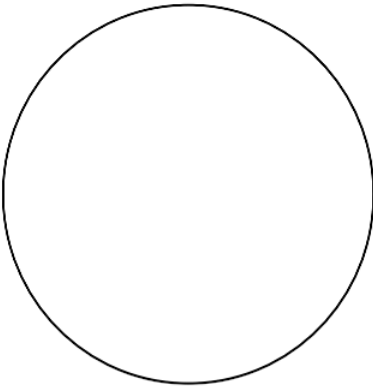
High power



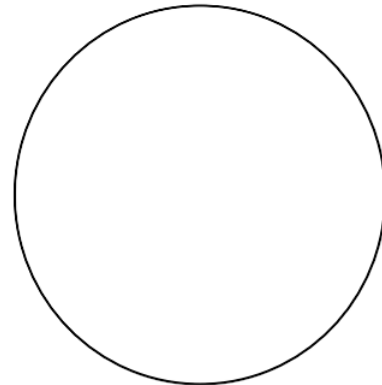
Total Magnification

Monocytes

Low power

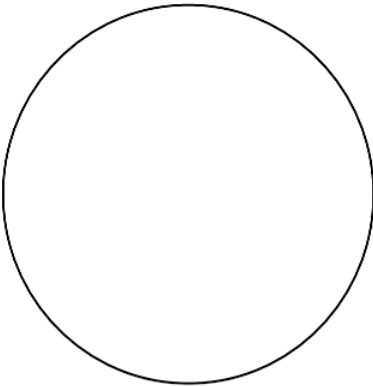


High power

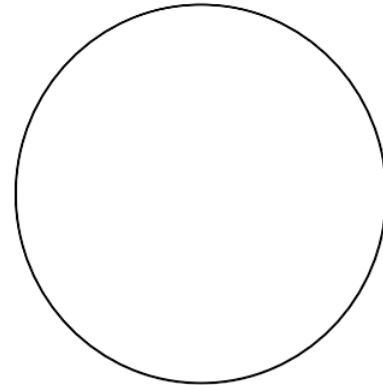


Total Magnification

Low power



High power



Total Magnification

Data

Count a total of 50 cells (or more!) and fill out the table below.

	Monocytes	Lymphocytes	Neutrophils	Eosinophils	Basophils
Number					
Percentage					
Expected percentage	3-7%	25-33%	55-65%	1-3%	<0.2%

Table 2: Student Data Table for Collecting White Blood Cell Counts

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12.3: Review Questions

Review Questions

1. What is the role of each of the cell types you saw today? Include erythrocytes and platelets in your answer.
 2. Which cells that we saw today have primary roles in the innate immune system? What about the adaptive immune system?
 3. Which cell type differentiates into innate immune system cells that are “professional” antigen-presenting cells?
 4. Can you tell the difference between B- and T-cells under the microscope? If not, what would you have to do to differentiate between them?
-

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CHAPTER OVERVIEW

13: Agar art

[13.1: Introduction](#)

[13.2: Procedures](#)

[13.3: Results](#)

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13.1: Introduction

Agar is a seaweed extract used to make liquids semi-solid at room temperature, a technique used for cooking in Indonesia, Japan and other Asian countries. In the late 1800's Angelina Hesse, the wife of Walther Hesse, suggested using the technique for isolating microbes in his work with Robert Koch. Because agar has a higher melting point than gelatin, agar plates will remain solid at 37° C, a common temperature for incubating bacteria.

Cultivating microbes on agar plates has become standard practice in microbiology. While not all microbes can be grown on agar plates, being able to study and experiment on the species that can grow has given scientists deep insight into microbial characteristics. When looking at growth on agar plate, the results will show a range of morphological characteristics depending on the species and growth conditions. Agar alone is not sufficient to grow microbes and agar plates need to contain added nutrients to grow. Different media have been developed over the last century to help grow, characterize and identify different types of microbes.

Visually, the growth of microbes on agar can be stunning. While often microbes we use in introductory lab classes grow as colorless colonies with smooth edges and defined growth patterns, the world is full of microbes that can grow with vibrant colors or in interesting patterns. Pigmentation occurs when a microbe produces a molecule that causes it to reflect a certain wavelength of light. In nature, pigmentation can be important for photosynthetic microbes that need to absorb certain wavelengths of light while reflecting others. Beyond pigmentation there are even microbes that can glow in the dark using a process called bioluminescence!

The diversity of the microbial world in terms of coloration and appearance has led to the development of a creative application for microbial growth on agar called **agar art**. Scientists, experts, and amateurs alike have used microbes to generate colorful patterns and depictions in artistic endeavors.

All agar art needs to follow the same principles to generate an intended design. The process can be a challenge, as often the artist cannot see the medium they are working with as they plate the microbes and have to wait for them to grow. Before starting, an agar artist needs to be familiar with aseptic techniques to prevent contamination of their work and make sure they are following biosafety procedures as they work. The type of media (nutrients, pH, etc.) and the temperature at which the plates will be incubated must all be carefully considered before starting.

As a design gets more complex, the artist may choose to use multiple different microbes in their work so that they can have different and more complex effects. There is a complication to using multiple species, the principle of competitive exclusion. The **competitive exclusion principle** states that one species will out-compete another when competing for the same resources, and thus it is typical for only one species to be able to grow in a given area. This means that you cannot mix microbes the way you might mix paint colors to get an even distribution of two different colors in the same area. Rather, one microbe will outgrow another if you inoculate them in the same spot. To avoid these complex interactions, microbes are typically inoculated in separate areas of the plate rather than on top of one another.



Butterfly Agar Art, also known as "microbial art" or "agar art," is a creative and visually appealing way to cultivate living microorganisms on agar plates to create artistic designs. This form of art combines science and artistry, using the natural pigments and growth patterns of various microorganisms to form intricate patterns and images. By: [Ajay Kumar Chaurasiya](#)



"Cell to Cell" by microbiologists Mehmet Berkmen and Maria Peñil from Massachusetts won the "People's Choice" award in the American Society of Microbiology's 2015 Agar Art Contest. (*Cell to Cell* by Mehmet Berkmen and Maria Peñil Cobo. American Society for Microbiology Agar Art Contest 2015, People's Choice / [Wikimedia Commons](#) / CC BY-SA 4.0)

WILL UPDATE LATER!

Sources:

<https://www.asianscientist.com/2016/01/columns/history-agar-microbiology-lab/>

<https://fems-microbiology.org/femsmicroblog-got-agar-say-thanks-to-angelina-hesse/>

Art sources:

https://commons.wikimedia.org/wiki/File:Butter_Fly_Agar_Art_with_Living_Microorganisms.jpg

https://commons.wikimedia.org/wiki/File:Cell_to_Cell.jpg

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13.2: Procedures

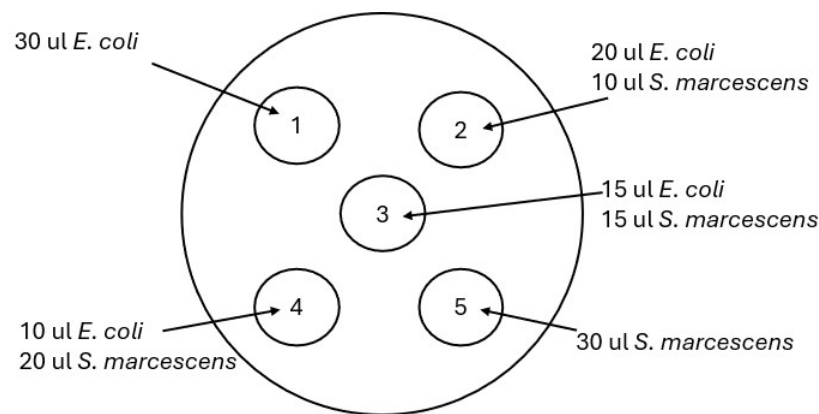
Materials:

- *E. coli*, *S. marcescens*, *M. luteus*, and other microbes provided by your instructor
- TSA plates – if hand drawing templates note that plates are 3.5" in diameter
- Bunsen burner
- Sterile toothpicks, inoculation loops
- Micropipette and tips

Procedure:

Part 1: Examining competitive exclusion

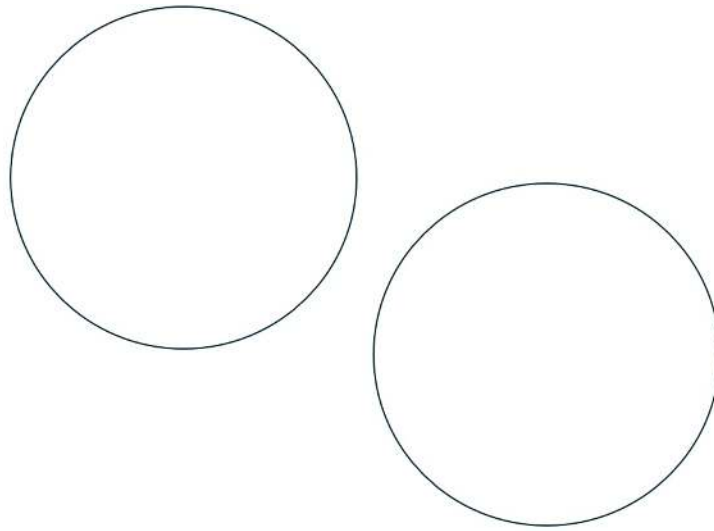
1. Label a TSA plate with your initials, date, and class number.
2. Using the template below, label each spot.



3. Pipette the indicated quantity of each strain onto the agar plate at the corresponding spot.
4. Set the plate aside and let the spots completely dry before flipping the plate over for incubation.
5. Incubate the plate for 24-48 h. at room temperature (~25 °C)

Part 2: Agar art

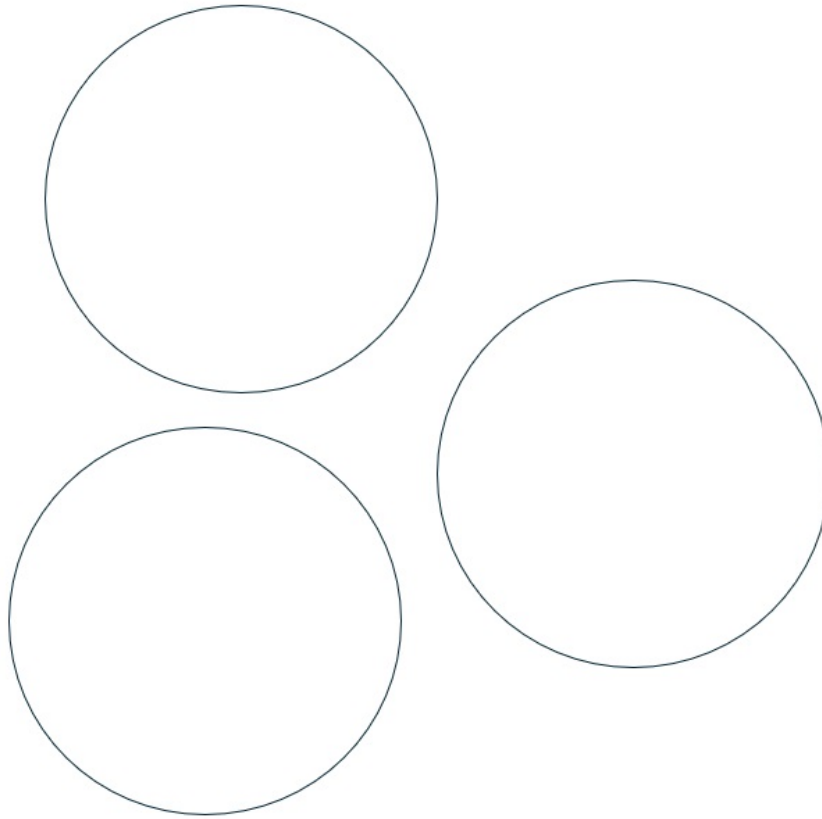
1. Sketch your designs onto the template below. Label which strain you intend to inoculate for each portion of your sketch.
2. Label two TSA plates with your initials, date, and class number.
3. Using sterile toothpicks, inoculation loops, or pipettes as needed for your design, inoculate the agar plate using aseptic technique.
4. Let the plate dry
5. Invert your plate and incubate it at room temperature (~25° C) for 24-48 hours



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13.3: Results

1. Draw out the results for each of your plates.
2. For each plate, write a short description of what the plate looked like and explain how the competitive exclusion principle did or did not affect the results.
3. Did your result match your expectations when you planned and executed your art piece?
4. What might you do differently if you were to do this again?



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Index

B

Bacterial Smear

[6.2: Procedures](#)

C

Capsule Staining

[6.1: Introduction](#)

D

disc diffusion test

[9.2: Procedure](#)

G

Gram staining

[6.1: Introduction](#)

P

Pasteurization

[10.1: Introduction](#)

S

Silver staining

[6.1: Introduction](#)

T

The Scientific Method

[2.1: The Scientific Method](#)

Glossary

Sample Word 1 | Sample Definition 1

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 - Title Page - *Undeclared*
 - 1.1: About the Author - *Undeclared*
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 - 3.4: Review Questions - *CC BY-NC-SA 4.0*
 - 3.5: Scientific Nomenclature - *CC BY-NC-SA 4.0*
 - 4: Introduction to Aseptic Techniques and Growth Media - *CC BY-NC-SA 4.0*
 - 4.1: Introduction - *CC BY-NC-SA 4.0*
 - 4.2: Procedure - *CC BY-NC-SA 4.0*
 - 4.3: Results - *CC BY-NC-SA 4.0*
 - 4.4: Review Questions - *CC BY-NC-SA 4.0*
 - 4.5: Examples of Bacterial Growth Characteristics in Broths, Slants and Plates - *CC BY-NC-SA 4.0*
 - 5: Quantifying bacterial cultures - *Undeclared*
 - 5.1: Determination of Bacterial Numbers - *CC BY-NC-SA 4.0*
 - 5.2: Introduction to Micropipetting - *CC BY-NC-SA 4.0*
 - 5.3: Serial Dilution - *CC BY-NC-SA 4.0*
 - 5.4: Procedures - *Undeclared*
 - 5.5: Results - *Undeclared*
 - 5.6: Dilution Worksheet and Problems - *CC BY-NC-SA 4.0*
 - 6: Preparation of Bacterial Smears and Introduction to Staining - *CC BY-NC-SA 4.0*
 - 6.1: Introduction - *CC BY-NC-SA 4.0*
 - 6.2: Procedures - *CC BY-NC-SA 4.0*
 - 6.3: Results - *CC BY-NC-SA 4.0*
 - 6.4: Summary of Common Bacterial Staining Techniques - *CC BY-NC-SA 4.0*
 - 6.5: Review Questions - *CC BY-NC-SA 4.0*
 - 7: Metabolic Activities of Bacteria - *CC BY-NC-SA 4.0*
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- 10.3: Results - *CC BY-NC-SA 4.0*
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 - 11.2: Solve the Outbreak - *Undeclared*
- 12: Immunology - *Undeclared*
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 - 12.3: Review Questions - *CC BY-SA 4.0*
- 13: Agar art - *Undeclared*
 - 13.1: Introduction - *Undeclared*
 - 13.2: Procedures - *Undeclared*
 - 13.3: Results - *Undeclared*
- Back Matter - *Undeclared*
 - Index - *Undeclared*
 - Glossary - *Undeclared*
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 - 6.2: Procedures - *CC BY-NC-SA 4.0*
 - 6.3: Results - *CC BY-NC-SA 4.0*
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 - 7.1: Introduction - *CC BY-NC-SA 4.0*
 - 7.2: Procedures - *CC BY-NC-SA 4.0*
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 - 13.1: Introduction - *Undeclared*
 - 13.2: Procedures - *Undeclared*
 - 13.3: Results - *Undeclared*
- Back Matter - *Undeclared*
 - Index - *Undeclared*
 - Glossary - *Undeclared*
 - Detailed Licensing - *Undeclared*
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