Part I: Scientific Methods

Introduction: Often as teachers we tell students what they need to know without helping them understand why they should know it. The following unit was designed to give students an understanding of how the acceptance of the scientific method led to better medical practices in the 1900's. Students will learn that well-accepted theories are ones that are supported by different kinds of scientific investigations. This unit addresses the NYS Living Environment Standard #1 "The central purpose of scientific inquiry is to develop explanations of natural phenomena in a continuing and creative process."

I. Overview: Concepts addressed

- How infectious diseases have affected history
- Koch's Postulates (what they are and how are they are still relevant today)
- Designing a controlled experiment (developing a hypothesis, defining independent and dependent variables, importance of using control groups for comparison, testing only 1 variable at a time)
- Organizing data in tables and graphs

II. Student Prior Knowledge and Skills Required

- No prior knowledge is required. Can be done in high school or middle school
- This would be a great unit to begin with early in the year to introduce scientific inquiry skills
- The experimental design matrix is a graphic organizer created by Dr. Nancy Elwyss of SUNY Plattsburgh. It can be used in any science course in which students are designing experiments.

III. Time Requirement

- 2-3 – 40 min. class periods depending on the pace of the instructor and reading level of the students
- 1-2 – 40 min lab periods depending on the level of the students

IV. Advance Preparation

- This first series of lessons requires a copy of Victoria E. Rinehart's book Portrait of Healing **ISBN # 0-925168-83-1**
- Graphing paper
- Student activity sheets and experimental design matrix (attached)

V. What is expected from students

- Student activity #1: Students read a passage from the book and answer focused questions
- Student activity #2: Students use the experimental design matrix to critically analyze an experiment
- Lab #1: Students design a controlled experiment.
- Lab #2: Students make a table for data entry and practice graphing data from a table.

VI. **Assessment**

- Prior to these lessons have students design an experiment to test a hypothesis (any simple hypothesis). This will act as a pre-assessment of their understanding of experimental design.
- After completing these lessons give them back their pre-assessment and using the experimental design matrix have students critique their own work and redo the assignment.
- The final revision of their experiment can be used as an indication of mastery.

Lesson #1: Medical Research Now and in the past

Objectives:

- Students will understand the importance of testing theories by learning about misconceptions → poor medical practices.
- Students will learn about 3 major diseases that have caused and continue to cause human suffering and death : tuberculosis, influenza, and pneumonia

Introduction and Input:

1. In order to gain a historical perspective students will pretend to be living in the 1800's
   a) give students numbers between 1 and 10
   b) have the 10's stand up → they represent the people that would have died of tuberculosis before the age of 40
   c) 1's stand up → represent people dying of influenza before 40
   d) 3's stand up → represent people dying of pneumonia before 40
   e) Ask students why the average lifespan of an American today is much higher than it was in the 1800's → increased sanitation, antibiotics, vaccines…

2. A brief description of each disease should follow. This can be accomplished by lecture or by student reading and highlighting of the the following passages:

   a) During the 19th century, TB claimed more lives in the United States than any other disease. But, with improvements in nutrition, housing, sanitation, and medical care during the first half of the 20th century, the number of cases and deaths dropped dramatically. In the 1940s and 1950s, with the introduction of antibiotic therapies for TB, the decline continued. By 1985, the number of cases had fallen to the lowest figure recorded in modern US history.
   However, TB re-emerged as a serious public health problem in the US, with more than 25,000 active TB cases reported in 1993, an increase of 14 percent since 1985. Between 1992 and 1998, the number of reported TB cases declined 31 percent. However, in addition to those with active TB, an estimated 15 million people in the US have latent TB infections that may develop into active TB at some time in their lives.

   The following are the most common symptoms for TB. However, each individual may experience symptoms differently.

   - cough that will not go away
   - fatigue
   - loss of appetite
   - loss of weight
   - fever
   - coughing blood
   - night perspiring

UNIVERSITY OF MARYLAND MEDICINE
(http://www.umm.edu/travel/tuberc.htm)

b) Influenza epidemics had been known to occur in large epidemics in the mid 1700's and the late 1800's. One of the worst plagues in modern times claimed 20 million people from 1918-1919. Although the symptoms of influenza were understood, and some basic concepts in epidemiology and disease prevention were emerging, the aggressive nature of this epidemic caught everyone by surprise. More people died (estimated 20-40 million worldwide) from the epidemic of 1918-1919 than died in World War 1 (1914-1918).
Many patients reported being ill in the morning, demonstrated a bluish-purple complexion by noon-time, and were dead by evening. By December, hundreds of thousands had died in the U.S. These deaths have been attributed to a strain of influenza A.
http://www.hsc.wvu.edu/resource/mbim/Flynn/StructureAntigenicity/Orthomyxoviruses.htm

c) A child with pneumonia in the 1800's had a good chance of succumbing to the bacteria. Today, with the help of two "miracle" drugs, people are more likely to survive and live without pain. The discoveries of aspirin and penicillin drastically changed the face of modern medicine.
http://inst.augie.edu/~jjrobins/aspirin.html

Student Activity #1: In the late 1800's a man named Edward Livingston Trudeau entered medical school read pages 6 and 7 ((Portrait of Healing), Rhinehart) to gain a perspective on what it was like to go to medical school in the US at that time in history. Then answer the following questions:

1) What were the 4 things that Trudeau had to do in order to become a physician?

Pay a $5 entry fee to the college
Attend 2 or more lecture courses at the college
Pass a brief oral examination which each professor gave members of the graduating class
Spend 3 years as a student working under a reputable physician (paying the physician $100 each year of service)

2) How much time did he have to spend in the lab?

None

3) What was he taught about tuberculosis?

It was non-contagious, incurable, and inherited

4) How might this belief about tuberculosis, have influenced his treatment?

He might not have attempted to treat them at all, he would not have taken precautions with family members or himself to prevent infection and might not have looked for disease causing organisms.

Day 2 Lesson: Experimental Design

Objectives:

- Students will learn about the importance of using the scientific method by learning about misconceptions about diseases that were passed on without ever being tested.
- Students will learn how to identify the independent variable and the dependent variable in an experiment. They will also learn the importance of testing only one variable at a time.
- Students will learn about controlled factors in an experiment and understand the importance of having control groups.
- Students will also be exposed to Koch's Postulates.

Introduction and Input:

In 1884 German scientist Robert Koch developed a scientifically sound way of determining whether a disease was caused by an organism (such as a bacterium, virus, or other)
What was later called Koch's postulates is still used by scientists today when looking for causes of new diseases (such as AIDS)

Koch's postulates

1. **The microorganism must be found in all cases of the disease.**
2. **It must be isolated from the host and grown in culture.**
3. **When injected into another host it must cause the same disease.**
4. **You should be able to isolate the same microorganism again from the newly infected host.**

Using these steps Koch was able to identify a rod shaped bacteria associated with anthrax and a different rod shaped bacteria from tuberculosis patients.

Remember what the physicians of this time were being taught about TB.
"It was non-contagious, incurable, and inherited"

Now for the first time scientific methods were being used to prove that it might be caused by a 'germ' and that it is contagious!

Student Activity #2

1) Now read Dr. Trudeau's reaction to these exciting new discoveries (page 23 and 24 **- Portrait of Healing**)

2) Using the attached experimental design lab fill in the blanks for Dr. Trudeau's experiment.

3) While Dr. Trudeau's experiment provided some very useful results; there are some things that he could have done to make them more informative and more reliable. What are some things he could have done differently:

He tested too many variables at a time (food, air quality, space, and light)
He only tested on rabbits
He didn't have an objective measurement of health, just his opinion on how they looked

4) Choose just 1 variable to test and design a controlled experiment to determine its affect on this disease in rabbits. Use the design matrix to describe your experiment. Note that rabbit health can be measured in many different ways make sure that you choose a dependent variable that is easy to measure (such as: weight, breathing rates, survival, number of bacteria found in the lungs…)

Lab #1 Experimental Design

Scientific inquiry involves the testing of proposed explanations involving the use of conventional techniques and procedures. In this lab you will devise ways of making observations to test proposed explanations.

Below are the major steps involved in testing a proposed explanation (hypothesis). [Note: It is important to remember that to avoid bias in an experiment it is important to **repeat experiments**, use **a large sample size**, and data collection needs to be **objective**.]

1) **Title of experiment** = What are we trying to figure out?
    a. Ex: "The effect of the independent variable on the dependent variable on the organism being tested"

2) **Hypothesis** = What you predict will happen during the experiment (hypotheses are predictions based upon both research and observations
    a. Ex: "If you do this, then this will happen."

3) **Independent variable** = What you are testing or changing in your experiment
    a. [Note: you can only test one independent variable at a time]
    b. The independent variable goes on the X axis and it is usually the first column in a data table
    c. Remember to include units on graphs and tables.
    d. Ex: Time, temperature, and pH are common independent variables

4) **Dependent variable** = What you are measuring
    a. Dependent variable always goes on the y axis and is usually on the right hand side of a data table
    b. Remember to include units on graphs and tables.

5) **Procedures** = Describe as completely as possible the steps involved in setting up the experiment, including how and when you will make your measurements. Describe the groups that you will be setting up (including a proper control). State what you will measure and how you will measure it.

6) **Controlled factors** = things that it is important to keep the same during the experiment (**everything** except the variable being tested must be **treated equally**)

7) **Control group** = the group that is used as a standard for comparison in the experiment. Usually the group that does not get exposed to the independent variable

When designing an experiment use the attached design matrix as a guide to make sure you've included and you understand the major parts of a controlled experiment.

Experimental Design Matrix

Title of the experiment:

The effect of *the environment* on *disease symptoms*

in *TB infected rabbits*

Hypothesis: *It is believed that if given plenty of rest, fresh air, and food a TB infected organism will have fewer disease symptoms than a TB infected organism living in a poor environment.*

Independent Variable: The variable being tested

The environment is the variable being tested but note that there are more than one variables being tested here (amount of food, fresh air and amount of light)

Levels of independent variable being tested	*Best environment*	*Worst environment*	*Worst environment no TB*		
# of repeated trials	*5*	*5*	*5*		

Dependent Variable: The thing being measured

Overall health is being measured but note that his results are subjective not objective

Procedures: How and when you will measure

Note that it would be difficult to repeat his experiments exactly because they weren't described in detail students should be encouraged to include more detail than is recorded here.

Controlled Factors (List at least 5)

If students were to do this experiment - things that they should include as controlled factors include: Same type, age and sex of all rabbits being tested and infecting rabbits with the same batch of TB using the same amount and same technique.

Control Group:

Very important to include the worst conditions no disease group! Without it you wouldn't know if the effects seen were from the conditions alone or the disease and the conditions together.

Lab #2: Organizing Data Using Tables and Graphs

Objective:

- Students will learn how to organize data into a table.
- Students will learn the basic components of designing a line or bar graph.
  - How to label the axes, include a descriptive title, and provide appropriate scales for data.

Materials needed:

Pencil (not pen)
Graph paper

Introduction and input:

I. Designing a table

a.) Table should include a title that describes the independent variable and the dependent variable.

b.) The first row of any table should include headings describing what will be in each column. (ALWAYS REMEMBER TO INCLUDE UNITS IN THE HEADING)

c.) Columns on the right are used for putting data in (dependent variable or the thing being measured)

Title: The effect of temperature on seed germination in carrots		
Day of observation	**Total number of seeds that germinated**	
	10 (˚C)	**20 (˚C)**
7	0	5
10	20	35
15	40	70
20	45	80

Student activity

Design a data table below for the experiment that you designed. You don't have to fill in any data because you won't actually do the experiment.

II. Making a graph

a.) The independent variable (the one that you control) always goes on the X axis
Example: the days of observation

b.) The dependent variable (the one that you measure) always goes on the Y axis
Example: the number of seeds that germinated

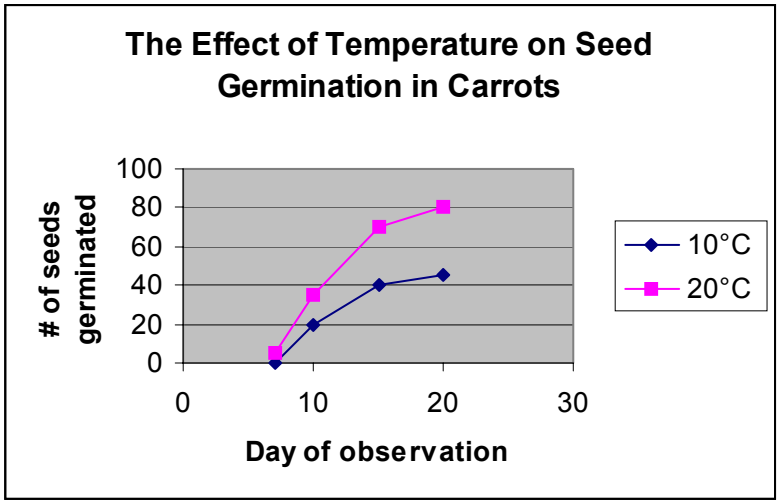
c.) REMEMBER to include UNITS on each axis

d.) Marking an appropriate scale (Use the 1,2,5 rule)

1) circle the highest # (ex: 80 is the highest number of seeds germinated)
2) circle the lowest # (ex: 0 is the lowest number of seeds germinated)
3) Both of those numbers have to fit on your graph!!!
4) Now count by 1's for each line
5) If the numbers won't go high enough Count by 2's
6) Then try counting by 5's
7) If the numbers still won't go high enough try 10's, 20's, 50's, 100's…

Student Activity

Using the data from the table below design a line graph comparing the number of seeds germinated at 10°C to the number at 20°C. Remember to include units.



Student Activity #1: In the late 1800's a man named Edward Livingston Trudeau entered medical school read pages 6 and 7 ((Portrait of Healing), Rhinehart) to gain a perspective on what it was like to go to medical school in the US at that time in history. Then answer the following questions:

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2) How much time did he have to spend in the lab?

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3) While Dr. Trudeau's experiment provided some very useful results; there are some things that he could have done to make them more informative and more reliable. What are some things he could have done differently?

_____

_____

_____

_____

4) Choose just 1 variable to test and design a controlled experiment to determine its affect on this disease in rabbits. Use the design matrix to describe your experiment. Note that rabbit health can be measured in many different ways make sure that you choose a dependent variable that is easy to measure (such as: weight, breathing rates, survival, number of bacteria found in the lungs…)

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2) **Hypothesis** = What you predict will happen during the experiment (hypotheses are predictions based upon both research and observations
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   d. Ex: Time, temperature, and pH are common independent variables

4) **Dependent variable** = _____
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When designing an experiment use the attached design matrix as a guide to make sure you've included and you understand the major parts of a controlled experiment.

Experimental Design Matrix

Title of the experiment:

The effect of _____ on _____

in _____

Hypothesis:

Independent Variable: The variable being tested

Levels of independent variable being tested					
# of repeated trials					

Dependent Variable: The thing being measured

Procedures: How and when you will measure

Controlled Factors (List at least 5)

Control Group:

Experimental Design Matrix

Title of the experiment:

The effect of _____ on _____

in _____

Hypothesis:

Independent Variable: The variable being tested

Levels of independent variable being tested					
# of repeated trials					

Dependent Variable: The thing being measured

Procedures: How and when you will measure

Controlled Factors (List at least 5)

Control Group:

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Materials needed:

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Introduction and input:

III. Designing a table

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Student activity

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IV. **Making a graph**

a) The independent variable (the one that you control) always goes on the X axis
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Student Activity

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Part II: Standard Laboratory Techniques

Introduction: In addition to demonstrating the performance indicators relating to scientific inquiry described in NYS Living Environment Curriculum Standard 1, students need to develop proficiency in certain laboratory skills in order to successfully conduct investigations in biological science. Part II contains 2 labs (a dilution / solution lab and an electrophoresis lab). The dilution / solution lab should be completed in 1—40min. lab period and the electrophoresis lab will require 2 (one to run the gel and one for the assessment. Included also is an extension of the dilution / solution intended for AP students. With the extension you may need an additional period to complete the dilution / solution lab.

I. Overview: Students will be analyzing DNA by gel electrophoresis, but before they begin they must make up certain solutions. As a result of these lab experiences students will:

- Have a better understanding of how to make
  1. A percent solution
  2. A dilution of a stock solution
- Practice using graduated cylinders and balances for measuring
- Understand the uses of gel electrophoresis as a way to separate molecules of different sizes and charges.
- Become more familiar with lab safety techniques

II. Student prior knowledge and skills required

- Students should be able to measure using either a triple beam balance or electronic balance
- Students should have basic math skills to be able to solve for x in a simple proportion

III. Time required:

- 3 --- 40 minute lab periods
- (optional lab for AP may require more time)

IV. Advance preparation

- Within each lab is a more detailed list of materials / lab station but the minimum requirements for all of the labs in this sections include:
  1. *Calculators and pencils*
  2. *10X TBE buffer (Fisher # BP1333-1)*
  3. *agarose*
  4. *beakers and graduated cylinders*
  5. *balance or scale and weigh paper*
  6. *magnetic stir bars and plate with heat (or stir plate and a separate heat source)*
  7. *DNA source (Edvotek)*
  8. *Methylene blue*
  9. *Electrophoresis chamber, casting tray with comb, and power source*
  10. *P20 microliter pipettor with tips for loading DNA samples*
  11. *Red and blue food coloring (for the optional extension lab)*

12. Distilled water and tap water

V. What is expected from students

- Lab #3 handout (dilutions and solutions) –
  1. students will solve mathematical equations using examples on the handout
  2. students will make a 1%agarose solution and dilute a 10XTBE solution to make a 1XTBE solution to be used in electrophoresis lab
- Lab #3 (optional extension for AP)
  1. Students will do dilution calculations
  2. Students will make a series of dilutions and use standard solutions to assess their work
- Lab #4 (electrophoresis handout)
  1. Students will pour a gel into a casting tray
  2. Students will practice using a microliter pipettor to measure small volumes
  3. Students will load DNA onto the gel
  4. Students will make and record observations based on the results of the gel and answer simple questions in relation to gel electrophoresis.
  5. Students will take a post lab test to assess understanding

VI. Assessment

- All student work should be incorporated into a student lab notebook or folder and graded based on level of completion, level of participation and level of understanding (based on post lab test).

Lab # 3: Solutions, Dilutions, and Concentrations Lab

Objective: Part of any lab includes the mixing of reagents to make solutions to work with. It is very important that the solutions you make are at the correct concentration for your experiment to be repeatable. (**Remember the way to make any experiment more valid or believable is for anyone to be able to repeat it and get the same results!**) Today we will practice making percent solutions. After completing this lab you should be able to:

- Make a percent solution
- Make dilutions of a stock solution

VII. **Part I (Calculations)**

Introduction and input:

Percent solutions are based on the volume or weight of something in 100ml.

When mixing a solid with a liquid you use

1g of solid to make 100 ml of a 1% solution
10g of solid to make 100 ml of a 10% solution

When mixing a liquid with water use

1ml of the stock to make 100ml of a 1% solution
10ml of the stock to make 100ml of a 10% solution

Example #1

A solution that is 10% sucrose has 10 grams of sucrose in 100ml of solution.
Note: The sucrose will take up some space in the solution so the amount of water you add will be a little less than 100ml

Example #2

What if we wanted to make 500 ml of a 6% sucrose solution?
We will have to set up a proportion:

A 6% solution has 6 grams in 100ml or $\frac{6g}{100ml}$

Even if the volume is different than 100ml the ratio is the same so if we want to make 500ml we could set up a proportion $\frac{6\ g}{100\ ml} = \frac{x\ g}{500\ ml}$

$6 * 500 = x * 100$
$3000 = 100x$
$30 = x$

therefore you need 30 grams of sucrose to make 500 ml of a 6% solution

Problem #1

For next week's lab you will need 200 ml of a 1% agarose solution. Set up a proportion to determine how much agarose you will need.

1g/100ml = x/200
100x = 200
x = 2g
Therefore you will need 2 g of agarose

Note: Always check your answers! Do the numbers you got make sense for what you are doing. (For example if you are making more than 100ml of a 1% solution then your answer has to be greater than 1 g)

Example #3

A 70% ethanol solution would be made of 70ml of ethanol plus enough water to make 100ml

100ml – 70ml = 30ml of water
Therefore to make 100ml of 70% ethanol add
70 ml of pure ethanol and 30 ml of water.

A simple formula to use when mixing liquids to make a % solution is :

C = concentration
V = volume

What you start with = What you want to make
$C_1V_1 = C_2V_2$

Example #4

To make a 100ml solution of 10% ethanol from a stock that is 100% ethanol how much water would you add.

$$C_1V_1 = C_2V_2$$

$$100\%\ (x) = 10\%\ (100ml)$$

$$x = \frac{10(100)}{100}$$

$$x = 10ml$$

Therefore you would need to add 10ml of 100% ethanol to make the solution
The rest of the solution will be water

100ml – 10ml = 90ml of water

Problem #2

For next week's lab you need to make a 1% TBE solution from a stock that is 10% TBE. We will need to make 1L of solution (remember that 1L = 1000ml). How much of the 10% stock solution will we need to add and how much water will we need?

10%(x) = 1% (1000ml)
x = 1000/10
x = 100ml of the 10% solution

1000ml – 100ml = 900ml of water

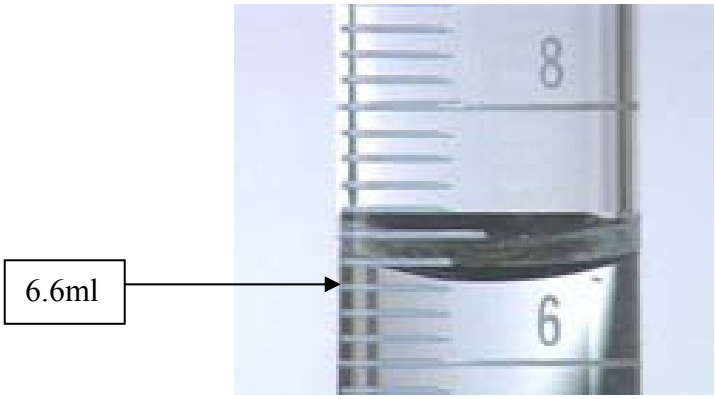
VIII.

IX. **Note: Always check your answers!** Do the numbers you got make sense for what you are doing. (For example if you are only making 100ml of a liquid then your answer has to be less than 100ml)

X. Part II (Making solutions)

Introduction and Input: When scientists do experiments it is very important to write down everything that they do? Why? *So they or someone else can repeat it exactly the same way* When you do anything in this lab it will be important to write down everything you do. In this part of the lab we will make the solutions we talked about in part I of this lab. There are a few things we need to remember about weighing measuring and mixing chemicals.

- Always use a graduated cylinder when measuring volumes of liquids. (Note: beakers often have numbers on them but they are not accurate enough to use when measuring)
- Always measure from the bottom of the meniscus (curved surface of a liquid)



Copied from http://www.middleschoolscience.com/meniscus.jpg

- When weighing solids use a triple beam balance or electronic scale
- Heat often helps solids go into solution
- Use extreme caution when heating liquids

http://www.usoe.k12.ut.us/curr/science/phillips/safety.gif

- Always write down the exact measurements that you used and label all solutions

Part II Lab Techniques Teacher's guide p.5

Materials at your lab station
Distilled water (1L)
1L and 500ml beakers
1L and 500ml graduated cylinders
Balance or scale
1 sheet weigh paper
scoop, spoon, or spatula to measure agarose
10X TBE (tris-borate-EDTA) (Need at least150ml)
Agarose (Need at least 2g)
1L Flask or bottle with a stopper
500ml Flask or bottle with a stopper
Magnetic stir bar and plate with heater
Pot holder for handling hot liquids
Sharpie and tape for labeling

Note: A brief explanation of 1X and 10X might be necessary before continuing. 1X refers to the standard usable concentration of a solution and 10X would be 10 times more concentrated than the standard.

Solution #1 Make 1L of a 1X TBE solution

1) First add a stir bar to your beaker

Substance to add	Amount needed	Amount added
10X TBE	100ml	
Distilled Water	900ml	

2) Add both substances to the beaker
3) Mix with the stir bar
4) Then pour the solution into a flask labeled 1X TBE with the date and your group name (store at room temperature for later use)

Solution #2 Make 200ml of a 1% agarose solution in TBE

Note: It is important that you use 1X TBE to make this solution not water!!

1) Put a stir bar into a 500 ml beaker and add the following:

Substance to add	Amount needed	Amount added
1X TBE	150 ml	
Agarose	2g	

2) Mix the above ingredients on the stir plate with low heat until all of the agarose has dissolved.
3) Pour the liquid into the 500ml graduated cylinder (CAUTION: Liquid will be hot use pot holders and pour carefully!!!)
4) Add enough 1X TBE to bring the volume up to 200ml
5) Pour back into the beaker and stir with stir bar again until thoroughly mixed
6) When mixed carefully pour into the 500ml flask and label 1% agarose with the date and your group name (store at room temp for later use)(note agarose should become solid as it cools)

Part III (Dilution Laboratory) *Note: This additional lab was designed for AP students and might be difficult for regular ed.*

Introduction and Input: The purpose of this lab is for you to become familiar with how to make and calculate a series of dilutions.
Making a 1:10 dilution means that 1 part from your solution will be diluted with 9 parts water.

Example: If you were to make a 200ml of a 1:50 dilution of solution A you would use a proportion to determine how much of A to add

1/50 = x/100
50x = 100
x = 2ml of solution A

If you need 2 ml of solution A you will need 98ml of water to make 100ml of solution

Practice:

Materials at your lab station

Tap water
Food coloring (red and blue)
P200 pipetor and pipet tips
100ml graduated cylinder
4 -100ml beakers
Sharpie for labeling

Procedure:

1) Add 200ul of red food coloring to 100ml of water and place in a beaker labeled solution A
2) Add 200ul of blue food coloring to 100ml of water and place in a beaker labeled solution 1
3) Make 200ml of a 1:10 dilution from solution A and label this solution B

Solution A add *20 ml*
Water add *180 ml*

Show your work:

What is the final dilution factor for this solution? Ans. *1:5000 dilution (note: solution A is a 1:500 x 10 gives you a final dilution of 1:5000)*

4) Make 100ml of a 1:50 dilution from solution 1 and label this solution 2

Solution 1 add *2 ml*
Water add *98 ml*

Show your work:

What is the final dilution factor for this solution? Ans. *1:25,000 dilution (note: solution 1 is a 1:500 x 50 gives you a final dilution of 1:25,000*

5) Make 100ml of a 1:20 dilution of solution B and label this solution C

Solution B add *5 ml*
Water add *95 ml*

Show your work:

What is the final dilution factor for this solution? *1:100,000 (note: solution B is a 1:5000 x 20 gives you a final dilution of 1:100,000*

6) Make 100ml of a 1:5 dilution from solution 2 and label this solution 3

Solution 3 add *20 ml*
Water add *80 ml*

Show your work:

What is the final dilution factor for this solution? *1:125,000 dilution of food coloring(note: solution 2 is a 1: 25,000 x 5 gives you a final dilution of 1:125,000*

7) Compare your solutions with the stock solutions in the front of the room.

Note for teachers: Prepare all of the solutions ahead of time and label them with the final dilution factor so that students can compare the colors they got from their dilutions with yours.

Lab #4: Gel electrophoresis

Objective:

In this lab students will run DNA fragments of different sizes on a gel and observe their migration patterns to determine whether smaller DNA fragments travel faster or slower towards a + charge than larger fragments. This is an inexpensive lab that can be done with minimal equipment and does not require the use of kits. This lab was designed for any high school level biology class (not just AP) and does not require prior knowledge of restriction enzymes). Prior knowledge of DNA structure is recommended.

Reagents and materials:

Gel

Most commonly used gels are made with polyacrylamide or agarose. (Polyacrylamide is toxic and should be avoided when working with students.) Agarose has a larger pore size and is used to separate nucleic acids, large proteins, and protein complexes. Agarose can be purchased from a wide variety of sources, can be used at varying concentrations, and is relatively inexpensive.

For these experiments I prepared a 1% agarose gel in 1X TBE buffer. *Note: It is important to make your gel with TBE buffer and not water. The salts in the TBE buffer are necessary for the current to flow evenly through the gel.*

Agarose (Fisher scientific #BP164-25 (25g)~$47.25) *(enough for 25(100ul size) gels)*

Buffer

While there are some bufferless gel systems on the market (see www.invitrogen.com), they are a little more expensive than normal gels that are run in a liquid buffer. Bufferless gels are faster than conventional gel electrophoresis methods and therefore might be of interest to teachers trying to run a gel within a 40 min. lab period. Currently a package of 18 gels can be purchased for about $135. This would also require a one time purchase of the power base.

Conventional gel electrophoresis labs require the use of a basic pH buffer. The most commonly used buffer is TBE (Tris/Borate/EDTA). Below is a recipe for TBE buffer but I recommend purchasing a ready to use 10X solution from Fisher scientific and diluting it 1 to 10 prior to use. It stores for extremely long periods of time (up to a few years) and is very reliable.

10X TBE buffer (Fisher scientific #BP1333-1, (1L bottle)~$35 *(enough for at least 25 runs depending on the size of your chamber).*

---OR---

To make a 1L batch of a 10X TBE solution

Amount (g)	Reagent	Molarity	FW	Source
107.8	Tris Base	0.89	121.10	Fisher BP152-1
55.0	Boric Acid	0.89	61.83	Fisher BP168-500
9.3	EDTA·$2H_2O$	0.025	372.20	Fisher BP120-500

Might have to heat up to get the EDTA into solution

pH should be (8.0-8.5) filter through a 0.45u filter and store at room temp.

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DNA source

DNA ladders and markers are composed of DNA fragments that have been cut into various lengths. They can be purchased from a variety of sources. In my experience DNA is very stable for long periods of time and can be stored for more than 1 year as long as you avoid freeze/thawing by storing in a freezer that doesn't have a defroster. If one is not available you can store it at 4˚C in a regular refrigerator.

If you are using DNA that doesn't contain a loading dye, you will have to use one when you load the DNA. The loading dye weighs it down and allows you to visually monitor the speed of migration. There are many types of loading dyes to choose from. I recommend using a 10X solution of 0.25% bromophenol blue in 60% glycerol. *[Note: (0.125g bromophenol blue and 30ml glycerol → 50ml (you might have to add a drop of NaOH to make it blue)(50ml is more than you will need in a lifetime)]*

Most DNA ladders come with the dye already added so you can disregard this step if you are using a purchased ladder or marker containing a dye.

1) **1kb DNA step ladder (1000-10,000) → 10 bands (Fisher scientific #PRG6941 (90ug at 0.3ug/ml) $77) (Comes with a loading dye.)** *Enough for 300 lanes if you load 10ul/lane*
2) EDVOTEK will also sell you ready to load DNA from their kits. For these experiments I recommend using markers of different sizes for comparison. For 40 lanes each order the following DNA samples (DNA comes at a concentration of 0.15ug/ul. I recommend loading 10ul or 1.5 ug for good staining. The more you add the bigger the band will be, but 10ul will give you very clear bands (see sample results):
   EDVOTEK kit #130 special component C for $15 (C →1 band 3kb)
   And

XI. **EDVOTEK kit #130 special component D for $15** (D→2 bands 3kb and 1kb)

Safety Note: When purchasing DNA for student use it is best to purchase DNA from non-human sources due to the possible contamination of viral DNA. The DNA from the EDVOTEK kit above has been isolated from plasmid DNA and is considered safe for student use.

XII. DNA Staining

When choosing a stain for your DNA bands you should stay away from the more commonly used ethidium bromide or crystal violet as both are carcinogenic and should be avoided when working with students. As a nontoxic alternative I suggest using a 0.002% methylene blue solution. The problem with using methylene blue is that you have to add more DNA than you would have to if using ethidium bromide but the nice thing about it is that you can see the bands in regular light and take nice pictures using a normal digital camera. When using methylene blue it is necessary to add at least 200ug of DNA to each well.

Prepare a 0.2% stock solution of methylene blue solution in 0.1X TBE (0.2g methylene blue / 100ml 0.1XTBE). When staining your gel dilute the stock solution of methylene blue 1:100 in TBE buffer.

25g of methylene blue is $18.40 from **Sigma #MB-1**

Caution – stains everything – fingers, clothes, bench tops…

Gel Electrophoresis Apparatus

Power supply

Any power supply that can run at 110v should be fine. A single gel running at 100volts will be ready within an hour. More expensive models that can run at 220 volts can be purchased for faster runs.

Carolina sells a 110v unit for $186 (ww-21-3672)

And a 220v unit for $228 (ww-21-3672B)

XIII. Chamber and casting trays

Chambers and trays can be purchased from many vendors. One possible source is Carolina biological supplies

The following chamber can hold 2 gels at one time capable of running 32 samples if you use 2 casting trays stacked on top of each other.

Carolina #ww-21-3668, $189

Extra casting trays and 8 well combs can be purchased

Carolina #ww-21-3655, $33 per set

Sample Equipment Costs:

Item	**Vendor and catalog #**	**Price**
Electrophoresis chamber	Carolina #ww-21-3668	$189.00
Extra casting trays and combs	Carolina #ww-21-3655	$ 33.00
Power source	Carolina #ww-21-3672	$186.00
	Total	**$408.00**

While initial setup cost of buying the electrophoresis chamber and power source is high most of the reagents are reusable and store for long periods of time. While it would be great to have many chambers and power sources – up to 32 students can participate with just one chamber and power source by stacking 2 gels on top of each other.

Sample reagent costs:

Item	**Vendor and catalog #**	**Price**	**~ # of gels or lanes**
25g Agarose	Fisher scientific #BP164-25	$47.25	25 gels
10X TBE buffer	Fisher scientific #BP1333-1	$35.00	25 gels
DNA ladder (optional)	Fisher scientific #PRG6941	$77.00	300 lanes
DNA Marker 1 band	EDVOTEK kit #130 special component C	$15.00	40 lanes
DNA Marker 2 bands	EDVOTEK kit #130 special component D	$15.00	40 lanes
Methylene blue (25g)	Sigma #MB-1	$18.40	Unlimited
	Total	**$207.65**	**20 gels**

Using all of the above reagents the total cost / gel is only about $10.38

Note: Prices reflect 2003 catalog prices from each of the following vendors:

Carolina Biological Supplies http://www.carolina.com/

Fisher Scientific www.fishersci.com 1-800 640-0640

Sigma www.sigma-aldrich.com 1-800-325-3010

Lab prep for Teacher

1) Prepare a DNA sample for each student labeled with either a 1,2,or 3

Tube	DNA	Vol. DNA to add	Loading buffer to add
1	Hyper ladder (lots of fragments)	10.2ul	2ul
2	(C) 1 fragment (3kb)	10.2ul	---
3	(D) 2 fragments (1kb and 3kb)	10.2ul	---
Note: Because of the small volumes it is helpful to do a quick spin in a microcentrifuge after making these tubes if one is available.			

2) Set up a lab station for each gel to be run. Each lab station will need:
   100 ml 1% agarose
   500 ml 1x TBE
   1- 8 toothed plastic comb
   1- gel casting tray and chamber with power source
   roll of masking tape
   1- p20 ul pipettor and pipet tips
   2 ml 0.2% methylene blue
   centrifuge for quick spin of eppendorfs (optional but helpful)
   DNA samples (1 for each member of the class)

3) *In a 40 minute period - students should be able to pour the gel, load the samples and begin to run the gel. As the gel will probably take about an hour to run you may have to do the staining yourself. (You can let the gel sit in the stain overnight in the fridge or just for an hour at room temperature. Follow with de-staining as needed)*
4) *Analysis of data: Bands should be easily seen and a digital image can be produced by taking a picture with any digital camera.*
5) *Safety concerns:*

a) *When using the hot agarose be sure to use pot holders and remove all caps or loosen them before putting into the microwave. Pressure will build up as you heat the solution.*
b) *When swirling the hot liquid steam will be released so it will be necessary to keep the bottle pointed away from your body.*
c) *Some gel casting trays are made of a less durable plastic and require cooling of the gel before pouring. Be sure to follow manufacturers advice before pouring hot gel. It may be necessary to wait until gel is luke warm (but still liquid)*

d) *If students are staining you may want to have them wear protective gloves and lab coat to prevent getting any of the stain on items of clothing*
e) *Use caution with all electrical wires and outlets, and avoid contact with the chamber until the power source has been disconnected*

Lab #3: Gel electrophoresis

Objective: Gel electrophoresis is a technique used to separate proteins and nucleic acid fragments based on the size and charge of the molecules. The mobility of a molecule through an electrical field will depend on the strength of the field, the net charge, size and shape of the molecule, and the concentration and temperature of the gel.

In this lab you will run DNA fragments of different sizes on a gel and observe their migration patterns to determine whether smaller DNA fragments travel faster or slower towards a + charge than larger fragments.

Materials / lab station:

Pot holders
100 ml 1% agarose
1- Gel casting tray
1- Electrophoresis chamber with power source (note: 2 lab groups can share 1 chamber and power source)
roll of masking tape
1- p20 ul pipetor and pipet tips
centrifuge for quick spin of eppendorfs (optional but helpful)

DNA samples (1 for each member of the class)

2-3 ml 0.2% methylene blue
1 spatula and one tupperware container
} For staining procedures if done during class

Procedure:

1) Make sure the lid on the agarose bottle is loose before putting it in the microwave oven and melt the 1% agarose solution in a microwave (1-3min.) so that it is completely liquid. Set the microwave oven for 1 min. swirl, than add 30seconds swirling each time, until the solution has completely melted **(make sure you handle the hot agarose carefully using pot holders as it will be very hot!)** *Note: a hot water bath can melt agarose if a microwave is not available.*
2) If your gel casting tray doesn't have removable ends you may need to put masking tape around the edges so that you can pour the gel in. Make sure there are no gaps for the hot gel to squeeze though.
3) Insert the comb and pour the hot gel into the casting tray filling it up to the top of the teeth-marks of the comb (note some casting trays will melt if the solution is too hot so you may need to wait until it cools follow instructions from the manufacturer).
4) Wait for the gel to harden (this should take about 20 min.)

5) During this time each student will practice using the ul pipetor with water and receive a DNA sample:
   a) All samples labeled #1 contain DNA ladders (pieces of DNA that have been cut up into 10 different size fragments of known lengths using specific restriction enzymes)
   b) Samples labeled #2 contain a marker that contains a fragment of DNA that is 3 kb long
   c) Samples labeled #3 contain 2 fragments one that is 1kb and one that is 3kb

Question # 1: What do you think will happen to each of the samples when we run them on the gel? Hint: DNA has a slightly negative charge.

Answers will vary but could include: DNA will move towards the + electrode

Smaller fragments will migrate farther on the gel than the larger ones

6) Remove the tape when the gel is solid and put into the electrophoresis chamber (be sure that the comb is closer to the negative (black) end
7) Fill the chamber with 1X TBE buffer making sure the gel is completely submerged and carefully remove the comb
8) Each student can now load 10ul of his/her sample in each well in order from 1-3
9) Attach the power-source cables into the chamber (being extremely careful to plug black into black and red into red)
10) Set the power-source at 100volts and start

Question #2. Describe what is happening.

Answers will vary but could include: buffer starts to bubble, dye is moving towards the + electrode

11) Run the gel until the loading dye band is about ½ inch from the bottom of the gel then turn it off and unplug the apparatus. (30 min. – 1 hour)

12) Staining the DNA ---
   a) Dilute the 0.2% methylene blue solution 1:100 in TBE buffer (you will need about 200ml so take 2ml methylene blue in about 98ml 0.1%TBE buffer)
   b) Remove the gel from the chamber with a spatula and put into a small tupperware container containing the 0.2% methylene blue solution
   c) Gel can be left to stain overnight at 4ºC or for 1 hour at room temp.

13) Destaining the gel---

a) To better visualize the bands it might be necessary to destain by putting the gel into a fresh tupperware container full of distilled water
b) The water can be changed several times until the bands are clear.

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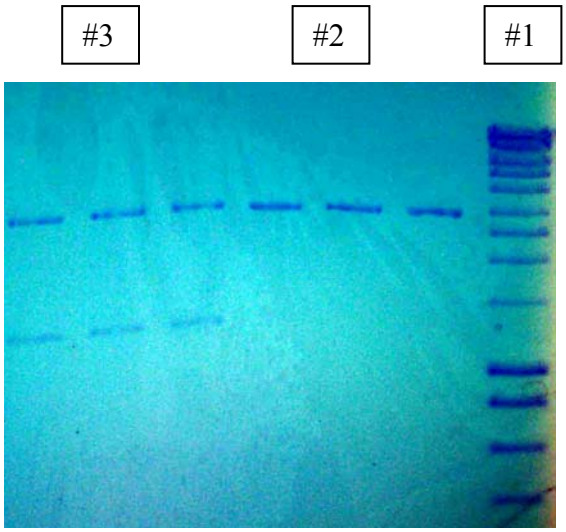
XIV. DAY 2

Results: Draw a picture of what your gel looks like being sure to label the lanes



This gel was obtained using 10ul or 1.5ug of Edvotek DNA / well. Using more DNA will give you bigger bands but for these purposes 1.5 ug works well. #3 represents component D and #2 represents component C from Edvotek kit #130

Conclusions: Based on your results which fragments travel the farthest through the gel and why?

The smaller the fragment the further the band traveled due to less resistance. Changing the concentration of the gel itself will change the rate of migration. A gel with less agarose will allow larger fragments to migrate faster. To test this theory students could design a lab using gels of varying concentrations while keeping all other parameters the same. They could then measure and record the distance traveled in a set amount of time.

Note: An optional activity would be to have the students determine the size of the fragments as unknowns by making a standard curve of the ladder fragments and plotting it on semi-log paper.

Lab # 3: Solutions, Dilutions, and Concentrations Lab

Objective: Part of any lab includes the mixing of reagents to make solutions to work with. It is very important that the solutions you make are at the correct concentration for your experiment to be repeatable. (**Remember the way to make any experiment more valid or believable is for anyone to be able to repeat it and get the same results!**) Today we will practice making percent solutions. After completing this lab you should be able to:

- Make a percent solution
- Make dilutions of a stock solution

XV. Part I (Calculations)

Introduction and input:

Percent solutions are based on the volume or weight of something in 100ml.

When mixing a solid with a liquid you use

1g of solid to make 100 ml of a 1% solution
10g of solid to make 100 ml of a 10% solution

When mixing a liquid with water use

1ml of the stock to make 100ml of a 1% solution
10ml of the stock to make 100ml of a 10% solution

Example #1

A solution that is 10% sucrose has 10 grams of sucrose in 100ml of solution.
Note: The sucrose will take up some space in the solution so the amount of water you add will be a little less than 100ml

Example #2

What if we wanted to make 500 ml of a 6% sucrose solution?
We will have to set up a proportion:

A 6% solution has 6 grams in 100ml or $\frac{6g}{100ml}$

Even if the volume is different than 100ml the ratio is the same so if we want to make 500ml we could set up a proportion $\frac{6\ g}{100\ ml} = \frac{x\ g}{500\ ml}$

$6 * 500 = x * 100$
$3000 = 100x$
$30 = x$

therefore you need 30 grams of sucrose to make 500 ml of a 6% solution

Problem #1

For next week's lab you will need 200 ml of a 1% agarose solution. Set up a proportion to determine how much agarose you will need.
Show all work:

Note: Always check your answers! Do the numbers you got make sense for what you are doing. (For example if you are making more than 100ml of a 1% solution then your answer has to be greater than 1 g)

Example #3

A 70% ethanol solution would be made of 70ml of ethanol plus enough water to make 100ml

100ml – 70ml = 30ml of water
Therefore to make 100ml of 70% ethanol add
70 ml of pure ethanol and 30 ml of water.

A simple formula to use when mixing liquids to make a % solution is :

C = concentration
V = volume

What you start with = What you want to make

$C_1V_1 = C_2V_2$

Example #4

To make a 100ml solution of 10% ethanol from a stock that is 100% ethanol how much water would you add.

$$C_1V_1 = C_2V_2$$

$$100\%\ (x) = 10\%\ (100ml)$$

$$x = \frac{10(100)}{100}$$

$$x = 10ml$$

Therefore you would need to add 10ml of 100% ethanol to make the solution
The rest of the solution will be water

100ml – 10ml = 90ml of water

Problem #2

For next week's lab you need to make a 1% TBE solution from a stock that is 10% TBE. We will need to make 1L of solution (remember that 1L = 1000ml). How much of the 10% stock solution will we need to add and how much water will we need?

XVI.

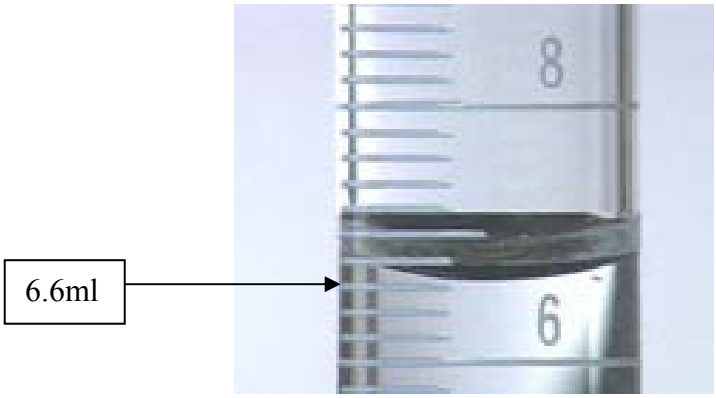
Note: Always check your answers! *Do the numbers you got make sense for what you are doing. (For example if you are only making 100ml of a liquid then your answer has to be less than 100ml)*

XVIII. Part II (Making solutions)

Introduction and Input: When scientists do experiments it is very important to write down everything that they do? Why?

_____ When you do anything in this lab it will be important to write down everything you do. In this part of the lab we will make the solutions we talked about in part I of this lab. There are a few things we need to remember about weighing measuring and mixing chemicals.

- Always use a graduated cylinder when measuring volumes of liquids. (Note: beakers often have numbers on them but they are not accurate enough to use when measuring)
- Always measure from the bottom of the meniscus



Copied from http://www.middleschoolscience.com/meniscus.jpg

- When weighing solids use a triple beam balance or electronic scale
- Heat often helps solids go into solution
- Use extreme caution when heating liquids

http://www.usoe.k12.ut.us/curr/science/phillips/safety.gif

- Always write down the exact measurements that you used and label all solutions

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Materials at your lab station
Distilled water (1L)
1L and 500ml beakers
1L and 500ml graduated cylinders
Balance or scale
1 sheet weigh paper
scoop, spoon, or spatula to measure agarose
10X TBE (Need at least150ml)
Agarose (Need at least 2g)
1L Flask or bottle with a stopper
500ml Flask or bottle with a stopper
Magnetic stir bar and plate with heater
Pot holder for handling hot liquids
Sharpie and tape for labeling

Solution #1 Make 1L of a 1X TBE solution

1) First add a stir bar to your beaker

Substance to add	Amount needed	Amount added
10X TBE	100ml	
Distilled Water	900ml	

2) Add both substances to the beaker
3) Mix with the stir bar
4) Then pour the solution into a flask labeled 1X TBE with the date and your group name (store at room temperature for later use)

Solution #2 Make 200ml of a 1% agarose solution in TBE

Note: It is important that you use 1X TBE to make this solution not water!!

1) Put a stir bar into a 500 ml beaker and add the following:

Substance to add	Amount needed	Amount added
1X TBE	150 ml	
Agarose	2g	

2) Mix the above ingredients on the stir plate with low heat until all of the agarose has dissolved.
3) Pour the liquid into the 500ml graduated cylinder (CAUTION: Liquid will be hot use pot holders and pour carefully!!!)
4) Add enough 1X TBE to bring the volume up to 200ml
5) Pour back into the beaker and stir with stir bar again until thoroughly mixed

6) When mixed carefully pour into the 500ml flask and label 1% agarose with the date and your group name (store at room temp for later use)(note agarose should become solid as it cools)

Part III (Dilution Laboratory) *Note: This additional lab was designed for AP students and might be difficult for regular ed.*

Introduction and Input: The purpose of this lab is for you to become familiar with how to make and calculate a series of dilutions.
Making a 1:10 dilution means that 1 part from your solution will be diluted with 9 parts water.

Example: If you were to make a 200ml of a 1:50 dilution of solution A you would use a proportion to determine how much of A to add

1/50 = x/100
50x = 100
x = 2ml of solution A

If you need 2 ml of solution A you will need 98ml of water to make 100ml of solution

Practice:

Materials at your lab station

Tap water
Food coloring (red and blue)
P200 pipetor and pipet tips
100ml graduated cylinder
4 -100ml beakers
Sharpie for labeling

Procedure:

1) Add 200ul of red food coloring to 100ml of water and place in a beaker labeled **solution A**

2) Add 200ul of blue food coloring to 100ml of water and place in a beaker labeled **solution 1**

3) Make 200ml of a 1:10 dilution from solution A and label this **solution B**

Solution A add _____
Water add _____

Show your work:

What is the final dilution factor for this solution? Ans. _____

4) Make 100ml of a 1:50 dilution from solution 1 and label this **solution 2**

Solution 1 add _____
Water add _____

Show your work:

What is the final dilution factor for this solution? Ans.
_____

5) Make 100ml of a 1:20 dilution of solution B and label this **solution C**

Solution B add _____
Water add _____

Show your work:

What is the final dilution factor for this solution?
_____

6) Make 100ml of a 1:5 dilution from solution 2 and label this solution 3

Solution 2 add _____
Water add _____

Show your work:

What is the final dilution factor for this solution?_____

Compare your solutions with the stock solutions in the front of the room.

Lab #3: Gel electrophoresis

Objective: Gel electrophoresis is a technique used to separate proteins and nucleic acid fragments based on the size and charge of the molecules. The mobility of a molecule through an electrical field will depend on the strength of the field, the net charge, size and shape of the molecule, and the concentration and temperature of the gel.

In this lab you will run DNA fragments of different sizes on a gel and observe their migration patterns to determine whether smaller DNA fragments travel faster or slower towards a + charge than larger fragments.

Materials / lab station:

Pot holders
Microwave oven
100 ml 1% agarose
1- Gel casting tray
1- Electrophoresis chamber with power source (note: 2 lab groups can share 1 chamber and power source)
roll of masking tape
1- p20 ul pipetor and pipet tips
centrifuge for quick spin of eppendorfs (optional but helpful)

DNA samples (1 for each member of the class)

2-3 ml 0.2% methylene blue
1 spatula and one tupperware container
} For staining procedures if done during class

Procedure:

1) Make sure the lid on the agarose bottle is loose before putting it in the microwave oven and melt the 1% agarose solution in a microwave (1-3min.) so that it is completely liquid. Set the microwave oven for 1 min. swirl, than add 30 seconds swirling each time, until the solution has completely melted **(make sure you handle the hot agarose carefully using pot holders as it will be very hot!)**
2) If your gel casting tray doesn't have removable ends you may need to put masking tape around the edges so that you can pour the gel in. Make sure there are no gaps for the hot gel to squeeze though.
3) Insert the comb and pour the hot gel into the casting tray filling it up to the top of the teeth-marks of the comb (note some casting trays will melt if the solution is too hot so you may need to wait until it cools follow instructions from the manufacturer).
4) Wait for the gel to harden (this should take about 20 min.)

5) During this time each student will practice using the ul pipetor with water and receive a DNA sample:
   a) All samples labeled #1 contain DNA ladders (pieces of DNA that have been cut up into 10 different size fragments of known lengths using specific restriction enzymes)
   b) Samples labeled #2 contain a marker that contains a fragment of DNA that is 3 kb long
   c) Samples labeled #3 contain 2 fragments one that is 1kb and one that is 3kb

Question # 1: What do you think will happen to each of the samples when we run them on the gel?

_____

_____

6) Remove the tape when the gel is solid and put into the electrophoresis chamber (be sure that the comb is closer to the negative (black) end
7) Fill the chamber with 1X TBE buffer making sure the gel is completely submerged and carefully remove the comb.
8) Each student can now load 10ul of his/her sample in each well in order from 1-3
9) Attach the power-source cables into the chamber (being extremely careful to plug black into black and red into red)
10) Set the power-source at 100volts and start

Question #2. Describe what is happening.

_____

_____

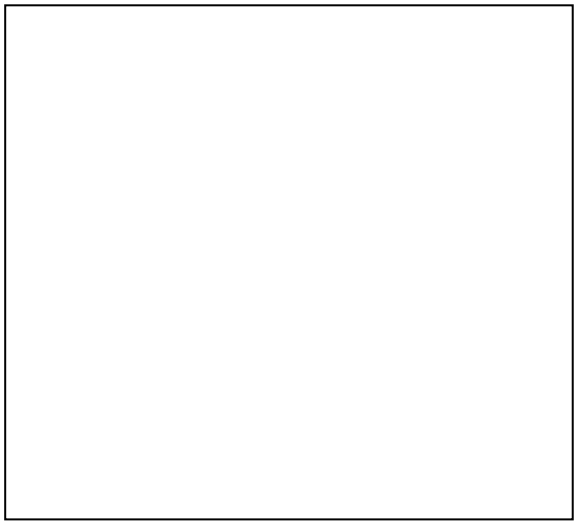
11) Run the gel until the loading dye band is about ½ inch from the bottom of the gel then turn it off and unplug the apparatus. (30 min. – 1 hour)

12) Staining the DNA ---
   d) Dilute the 0.2% methylene blue solution 1:100 in TBE buffer (you will need about 200ml so take 2ml methylene blue in about 98ml 0.1%TBE buffer)
   e) Remove the gel from the chamber with a spatula and put into a small tupperware container containing the 0.2% methylene blue solution
   f) Gel can be left to stain overnight at 4ºC or for 1 hour at room temp.

13) Destaining the gel---
   d) To better visualize the bands it might be necessary to destain by putting the gel into a fresh tupperware container full of distilled water
   e) The water can be changed several times until the bands are clear.

XIX. DAY 2

Results: Draw a picture of what your gel looks like being sure to label the lanes



Conclusions: Based on your results which fragments travel the farthest through the gel and why?

Post Lab Quiz

1) What should you use when measuring liquids?
   _____

2) If you have a solid that won't go into solution what can you do to help it?
   _____
   _____

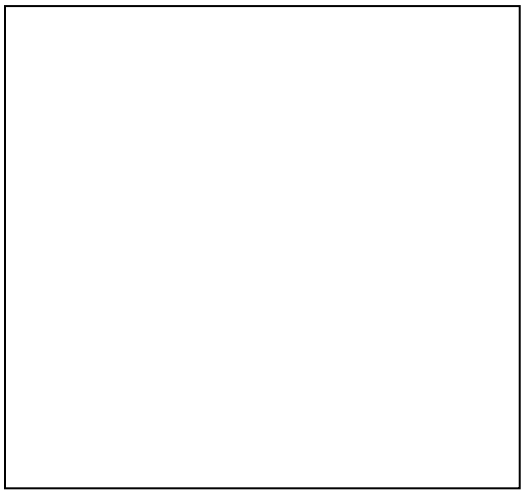
3) If we have a 10X stock solution and we want to make 200 ml of a 1X solution we will need to add _____ ml of the stock solution and we will have to add _____ml of water. (Show all work for full credit.)

4) List 3 safety procedures that should be used when heating a liquid in a test tube. _____

   _____

   _____

5) Draw a picture of a gel containing a DNA sample containing fragments that have been run under a current for 30min. The fragments are 1kb, 3kb and 10kb long. Label each of the fragments in the order that they would appear from top to bottom.



Part III: Basic Immunology

Introduction: This is an introductory unit on immunology. Important topics addressed include bacterial pathogens, vaccines, antibiotics, and cells of the immune system. After completing this unit it is still important to address viral pathogens through a viral disease system such as influenza or HIV (both of which are recommended as they are diseases addressed by many social studies curricula.)

I. Overview: Concepts addressed

- Definitions of pathogen, antigen infection, disease, immunity (natural and acquired), antibodies, antibiotics, vaccines, virulence, and bacterial plasmids
- Contributions by Louis Pasteur and Robert Koch in bacterial disease research
- The history of antibiotics and of vaccine development
- Extension computer activity for advanced placement students will introduce them to Pubmed resources, Genomics and the use of BLAST searches

II. Student prior knowledge and skills required

- Should follow a unit on classification as students should have a basic understanding of what bacteria are
- The extension activity requires a good understanding of DNA sequencing and protein synthesis
- Basic computer skills are required for the extension activity as students will be working with multiple windows open at the same time and copying and pasting information.

III. Time required

- 3 – 40 min. class periods depending on the pace of the instructor and reading level of the students
- extension computer activity should take about 2 – 40 min. class periods

IV. Advanced Preparation

- Student activity #1 requires a copy of the ASM News article Mikesell, Perry et. Al. "Plasmids, Pasteur, and Anthrax" VOL. 49, NO. 7, 1983
- Extension activity requires a computer lab with internet access

V. What is expected from students

- Student activity #1 involves students filling in guided notes sheets of definitions for immunity
- Student activity #2 requires students read the "Plasmids, Pasteur, and Anthrax" and answering related questions
- Student activity #3 has students reading "History of antibiotics" and answering related questions.
- Extension activity requires students to follow a series of steps using an online database to search for a sequence of bases and doing a BLAST search to find out if any other organisms contain similar sequences

VI. Assessment

- Each of the activities should be collected and graded and a final assessment should include a vocabulary quiz on frequently used terms.

Lesson 4: Treating bacterial disease

Objectives:

- Students will be able to define pathogen, immunity (natural and acquired), antigen, disease, antibiotic, and vaccine.
- Students will be exposed to the concept of immunological specificity.
- Students will gain a historical perspective of disease treatment by learning about the first antibiotics and vaccinations developed.

Introduction and Input:

- *Bacillus anthracis is a bacterium that causes a disease called anthrax.*
- *It is a very old disease that has been described in history since biblical times.*
- *Robert Koch conclusively proved that the bacteria caused anthrax by using a series of steps that has come to be known as Koch's Postulates.*
- *Louis Pasteur developed a vaccine that protected against anthrax in 1881 (or was it W.S. Greenfield in England?)*
- *It is a large, gram-positive, spore forming rod-shaped bacteria that can be grown under aerobic and anaerobic conditions*
- *Phenotypically and genotypically it is very similar to B. cereus and B. thuringiensis.*
- *Primarily a disease of cattle, sheep, goats, and wild animals, but humans can be infected by diseased animal products.*

Student activity #1: Students will receive guided notes sheets containing key vocabulary for a unit on immunology. Through direct instruction teachers will make sure that students write down and understand each of the definitions on their sheets.

Student activity #2: Students read "Plasmids, Pasteur, and Anthrax" and answer the questions using information from the article

Student activity #3: Students read "History of antibiotics" and answer the questions using information from the article.

Student Activity #1 Guided notes
Teacher's guide
Definitions for immunity

1) **Pathogen =** *A disease causing organism*
   Examples: *influenza virus, HIV virus, measles virus, tuberculosis bacilli, and anthrax bacilli*

2) **Antigen =** *The part of a pathogen that will cause an immune response*
   Examples: *a protein on the HIV virus, a sugar on the cell wall of bacteria*

3) **Infection =** *occurs when an organism is exposed to a pathogen*

4) **Disease =** *specific symptoms that an organism often has when infected with a specific pathogen.(Note: you can be infected without developing a disease)*

5) **Immunity** = *The ability of an organism to prevent a pathogen from causing a disease*

   Natural (innate) immunity = *refers to a type of immunity that* ***nonspecifically*** *prevents pathogens from entering a body or destroys pathogens* Examples of natural immunity = *skin acts as a barrier to prevent infection, some white blood cells (macrophages and neutrophils) kill anything that looks foreign*

   Acquired (adaptive) immunity = *immunity that involves killing* ***specific targets*** *and remembering how to kill those targets in the future.* Examples of acquired immunity = 1) *get infected-get sick-develp cells that kill only the specific target antigen- maintain memory to prevent getting sick from the same thing in the future 2) get immunized with a harmless form of antigen – develop cells that kill only specific target antigen – maintain memory to prevent getting sick from the same antigen in the future*

6) **Antibodies** = *Proteins found in the blood made by white blood cells that bind to antigens making them harmless. NOTE: antibodies are specific and bind only to the antigen that the organism was exposed to*

7) **Antibiotics** = *chemicals that kill bacteria (often produced by fungi)*

8) **Vaccines** = *harmless forms of antigen that trick your body into thinking that it has a disease → body responds giving it acquired immunity (Note: vaccines can be made to prevent viral diseases as well as bacterial diseases)*

9) **Bacterial plasmids** = *circular form of DNA found in bacteria that carries extra genes, not found in all strains of a bacterial species. (can carry genes for antibiotic resistance or virulence (disease causing))(can be easily transferred from one bacteria to another (naturally and manmade))*

10) **Virulence** = *describes the ability to transmit disease. A virulent strain of a bacteria is a strain that can cause disease.*

11) **Lymphocytes** = *white blood cells that are specialized to fight specific pathogens. There are 2 major types of lymphocytes*

    12) **T cells** = *lymphocytes that develop in the thymus and are involved in acquired immunity*

    13) **B cells** = *lymphocytes that produce antibodies when stimulated. Also part of the acquired response*

14) **Macrophages** = *Phagocytes that engulf pathogens and cell debris and break them down. Also capable of presenting antigens to T cells.*

15) **Platelets** = *blood clotting factors in the blood*

16) **Lymph** = *a fluid tissue containing white blood cells that drains liquid (containing foreign antigens) from the interstitial fluid. Lymph returns to the blood via lymph nodes*

Teacher's guide

Student Activity #2 Answer the following questions based on the information in the attached article "Plasmids, Pasteur, and Anthrax"

1) Name 3 things that have led to the decline of anthrax in humans in the US during the 1900's.

Vaccines for people who are at risk of exposure

Better working conditions

Less exposure to contaminated animal products

2) In 1877 Robert Koch was the first to demonstrate that *a specific bacteria was responsible for a particular disease.*

3) Louis Pasteur showed that organisms grown at high temperatures have *decreased virulence* and were capable of producing *immunity*

Pasteur wanted to know why bacteria grown at elevated temperatures was no longer virulent (disease causing). Thanks to advances in molecular biology and biotechnology, we now know the answer to this question.

4) Why can't heat attenuated bacteria cause disease? *The heat destroys a DNA plasmid that produces the toxin that makes people sick.*

XX.

"The History of Antibiotics"

What are antibiotics? Antibiotics are chemicals that will stop the growth of or kill certain types of bacteria. It is important to realize that different types of chemicals kill different types of bacteria. Therefore, an antibiotic that is good for one type of bacterial infection may have no affect on a different type of bacteria. In general antibiotics have no affect on viral infections and therefore should not be prescribed for things like the common cold or the flu, which are caused by viruses. The more we use antibiotics the more we select for antibiotic resistant strains of bacteria.

Many different types of fungi produce antibiotics. Scientists are constantly searching for new chemicals to use in the fight against pathogens. Many drug companies have research teams collecting rare plants, microbes and fungi from the rainforest and the ocean in hopes of finding and producing new antibiotics. Loss of biodiversity in these areas due to pollution, deforestation, and habitat destruction could mean the loss of millions of potential antibiotics and wonder drugs.

The first mass-produced antibiotic was discovered in 1929, by Alexander Fleming. He was studying staphylococcus bacteria in the lab by growing it up in petri dishes. Quite accidentally one of the plates became contaminated with a green fungus (similar to the green mold that grows on bread). He noticed that the bacteria couldn't grow in the presence of the mold. Many years later a chemical produced by that penicillium mold was mass-produced and used to treat a wide variety of diseases caused by bacteria.

Antibiotics, considered the wonder drugs of the 20th century, are becoming alarmingly less effective in the treatment of diseases. Many of the bacteria that were once killed by antibiotics have developed resistance to the antibiotics. In order to understand how bacteria have been able to adapt to changes in their environment, we must review a little of what we know about evolution. Evolution is a change in a population over time that occurs because:

- *There is variation within a population*
- *The organisms best adapted to the environment will survive*
- *The survivors of a generation will reproduce and pass those favorable genes on to the next generation*

Now if we put that into the context of a bacterial colony growing in the presence of an antibiotic, we realize that only the 1 or 2 bacteria that are naturally resistant to the antibiotic will survive and live to repopulate the

next generation of bacteria. Because bacteria reproduce so quickly, if one has resistance, it can become many in a relatively short period of time.

Unfortunately the widespread and often improper use of antibiotics over the past few decades has resulted in a serious problem. Many bacteria have become resistant to antibiotics that once killed them. Resistant bacteria make the treatment of infections very difficult. Ear infections in children, sinus infections, tuberculosis, and bacterial pneumonia are a few of the illnesses and conditions that have been affected by the threat of bacterial resistance. First choice and often second choice antibiotic therapies are no longer effective leading to fewer options for patients with dangerous and potentially life threatening diseases.

www.infectionspotlight.com/resistance/BacterialResistanceABriefHistory.jsp

Teacher's guide

Student Activity #3 Answer the following questions based on the information in the attached article "History of Antibiotics"

1) *What do antibiotics do?*
   Kill bacteria or prevent them from growing

2) *How are antibiotics obtained?*
   By isolating chemicals from plants, fungi, and microorganisms

3) *Why are drug companies buying up portions of the rainforest?*
   In hopes of finding new antibiotics from the huge variety of organisms found in rainforest ecosystems

4) *What did Alexander Fleming discover?*
   The antibacterial properties of penicillin

5) *What are 3 things that must occur in order for an evolutionary change to occur in a population?*
   a) variation within the population

   b) survival of the fittest

   c) reproduction of the fittest

Lesson #4 Extension for AP Biology

Scientific research: Anthrax sample exercise computer lab

Objective: This exercise was designed to teach students the importance of computers in analyzing, finding, and using data produced by the Human Genome Project.

- Students will use PubMed to gain information about an organism (Bacillus anthracis),
- will use the NCBI (National Center for Biotechnology Information) homepage to find out about and access DNA sequences from that organism,
- and will do a BLAST search to see if there are any other organisms with similar sequences.

After completing this activity students should have a greater appreciation and improved understanding of genetic diversity.

Procedure:

Part 1

Scientists often begin researching a topic by searching PubMed. PubMed is a free online service that can be used to browse online scientific journal references and abstracts. All of the information in the journal articles in the PubMed database are considered reliable because they have been peer reviewed. (Nothing gets published in a scientific journal unless it has been reviewed and scrutinized by a team of scientists in the field.) **In this exercise you will try to access information about the virulence of the species of bacteria responsible for causing anthrax.**

Question: What can you find out about the pathogenicity and virulence of Bacillus anthracis?

1) We will want to consult the primary literature to answer this question, so we will use PubMed to browse online journal references and abstracts.
2) Go to the NCBI (National Center for Biotechnology Information homepage on the web (www.ncbi.nlm.nih.gov/)
3) Click on the menu item that says 'PubMed' (you will find it at the top of the page just over the search box)
4) Enter 'anthrax virulence' into the text entry box next to 'Search PubMed for' and click 'Go'.
5) A list of your results will come up. We want something that looks like it has something to do with the cause of anthrax virulence.
6) Click on one of the articles to see an abstract of the paper.
7) Repeat the exercise using different search terms such as 'anthrax toxins', etc…

You'll soon find out that the more you learn about the topic – the more questions you have. You can now narrow your search to ask more specific queries.

After reading several abstracts you have obtained using PubMed, you should have some idea of how scientists search the literature for published information.

What did you learn about anthrax virulence?

_____

_____

_____

Part 2: (Scientists can also learn about the taxonomy of different organisms by using the NCBI homepage. When working with an organism it is essential to know as much as possible about that organism's phylogeny (evolutionary tree). Similar organisms have similar characteristics and similar genetic sequences.)
Question: What can you learn about the taxonomy of B. anthracis?

1) go to the NCBI homepage (www.ncbi.nlm.nih.gov/)
2) Click on the menu item that says 'Taxonomy'
3) Enter 'Bacillus anthracis' in the empty text box and click 'go' to initiate the search
4) A results page will come up listing the bacteria. Click on the blue link on the name of the bacteria to go to the page for Bacillus anthracis.
5) The lineage section at the top of the page describes the phylogeny of the bacteria. Click on the last subgroup 'Bacillus cereus group'.
6) This will show you a list of closely related bacteria species. Clicking on the names of the bacteria will bring up additional genetic information, including the DNA sequence if it has been done.
7) Notice that one of the bacterial species that resembles Bacillus anthracis is Bacillus thuringiensis (Bt). So what????

Read the article "Friend or Foe" to find out why this may be of concern.

1) What is Bt used for and why? *It is used as an insecticide because it produces toxins that kill insects*

2) What makes Bt, B. cereus, and B. antrhacis different? *Their chromosomal DNA is the same but they have or are missing different plasmids*

3) What conditions are necessary for plasmids to be swapped? *Bacilli must be in the growth phase*

4) Now try a google search (to look for more general information) to find out if and why they are spraying Bacillus thuringiensis in the Adirondacks.
What did you find? *Hopefully students will realize that they are spraying to kill black flies. I chose the Adirondacks because that is where we live I would suggest asking them to find it in their area.*

Part 3 Genomics is the comprehensive study of whole sets of genes and their interactions. Plasmids are extra pieces of bacterial genes that are not part of the chromosomes. They can replicate on their own and are capable of jumping from one organism to another. If you were a scientist that had identified a plasmid found in B. anthracis that seemed to be linked to virulence you might want to take a closer look at the genes and find out if any other types of bacteria had the same types of sequences.

In this part of the lab you will use an online database to search for the sequence of bases on the B. anthracis virulence plasmid pX02. You will then do a BLAST search to find out if any other organisms contain the same plasmid.

1) Go to the NCBI home page (www.ncbi.nlm.nih.gov/)
2) In the drop down box next to search select 'genome' then type in 'Bacillus anthracis virulence plasmid pX01'
3) You should get some references – select the blue reference # for the complete sequence for the plasmid (pX01)
4) If you are lucky you should now see a map of all of the genes on the plasmid –Let's look at one of the genes on the plasmid by selecting (pX01-142).
5) You should now see a blue line underneath what looks like a ruler representing the the gene you selected – select the entire sequence (by clicking on the blue line and then clicking on the + sign that should appear to the right) until the entire blue line is selected.
6) You will now see the sequence of bases that make up the gene on plasmid X01.
7) Notice the letters that appear below the bases. What do you think they represent? *The amino acids that each codon codes for.*

Now we can see if any other organisms have the same gene by doing something called a BLAST search.

8) *First we need to be able to copy the sequence. In order to do this we must go up into the search box at the top of the page and change the 'Display' to "FASTA" then select 'text' in the dropdown list next to 'send to' then select send to.*
9) Now you should see only the bases. Highlight the bases only by clicking and dragging and then select Edit Copy (make sure you don't highlight the first line which is a description of the sequence – just the bases)
10) Go back to the NCBI homepage. (www.ncbi.nlm.nih.gov/)
11) Finally we are ready to do a BLAST search!!! Select BLAST at the top of the page
12) Select 'Standard nucleotide-nucleotide blast'

13) In the Search box paste your sequence (under Options Expect change to 0.01) Then at the bottom of the page hit BLAST.
14) You will now have to wait while your computer searches all the databases for DNA sequences that are the same or similar to the one you are looking for.

RESULTS

You should end up with a list of hits after a few minutes. In order to look more closely at the sequences that matched click on one of them.

- Look at the number of identities: this will tell you what percent of the sequence is the same and what percent is different.
- Plus/plus means that the strands both read in the same direction
- Plus/minus means that they read in opposite direction
- You can also look at the bases together and see where they do and don't match up

Student Activity #1 Guided notes
Student copy page 1
Definitions for immunity

1) Pathogen = _____

Examples: _____,
_____

2) Antigen = _____

Examples: _____
_____

3) Infection = _____

4) Disease = _____
_____
_____

5) Immunity = _____

Natural (innate) immunity = _____
_____
_____Examples of natural immunity = _____
_____
_____

Acquired (adaptive) immunity = _____
_____
_____Examples of acquired immunity = 1) _____
_____
_____
_____
_____
_____

6) Antibodies = _____
_____
_____

7) Antibiotics = _____

8) Vaccines = _____
_____
_____
_____

9) Bacterial plasmids = _____
_____
_____
_____

10) Virulence = _____
_____

11) Lymphocytes = _____
_____

12) T cells = _____
_____

13) B cells = _____
_____

14) Macrophages = _____
_____

15) Platelets = _____

16) Lymph = _____
_____
_____

Student Activity #2 Answer the following questions based on the information in the attached article "Plasmids, Pasteur, and Anthrax"

1) Name 3 things that have led to the decline of anthrax in humans in the US during the 1900's.

_____

_____

2) In 1877 Robert Koch was the first to demonstrate that _____

_____

3) Louis Pasteur showed that organisms grown at high temperatures have _____

_____and were capable of producing _____

Pasteur wanted to know why bacteria grown at elevated temperatures was no longer virulent (disease causing). Thanks to advances in molecular biology and biotechnology, we now know the answer to this question.

4) Why can't heat attenuated bacteria cause disease? _____

_____

"The History of Antibiotics"

XXI.

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www.infectionspotlight.com/resistance/BacterialResistanceABriefHistory.jsp

Student copy page 4

Student Activity #3 Answer the following questions based on the information in the attached article "History of Antibiotics"

5) *What do antibiotics do?*

_____

___

6) *How are antibiotics obtained?*

_____

7) *Why are drug companies buying up portions of the rainforest?*

_____

_____

8) *What did Alexander Fleming discover?*

_____

9) *What are 3 things that must occur in order for an evolutionary change to occur in a population?*

- _____
- _____
- _____