Biology 110

Emerging Infectious Disease

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Spring 2007

Bio 110 Laboratory Manual
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Course Introduction

Biology 110: Emerging Infectious Disease
Spring 2007

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Class meetings:	Mon, Wed & Fri	9:20 & 10:25
Lab meetings:	See schedule for your section	

Biology 110 is designed for the non-science student. It is the intent of this course to expose you to current topics and policy issues in infectious diseases. Begin to understand how science works

- ❖ The role infectious disease in history
- ❖ To understand the role infectious diseases have played in public policy
- ❖ Be able to make observations and interpret data
- ❖ To become interested in reading and researching new biological threats
- ❖ To understand some scientific advances that will influence your life

Class policies:

1. Class attendance is mandatory for successful completion of this course. Excessive absences may be reflected in the final grade earned in this course. Attendance will be taking sporadically.
2. All work is due on time. Laboratory reports are due at the beginning of the lab period. If they are submitted after the lab has begun they will be considered late. Late work will be penalized 10% each day for 5 days. No work will be accepted after 5 days. No e-mail transmissions are acceptable.
3. Attendance in laboratory is mandatory. Failure to attend lab will result in a 10 points deduction from your overall points earned. In addition, if you miss a lab you cannot submit any written assignment from that week. However, if you miss a second lab (or more than that) that absence will result in a 25 point deduction.
4. Hard copies must be submitted for evaluation. E-mail copies are not acceptable.
5. The Honor Code will be upheld in this course. All written work must contain the signed Honor pledge when submitted.
6. Be prepared. Bring the appropriate hand outs to lecture and laboratory.
7. You are expected to be an active participant in the laboratory.
8. There will be NO make-up exams in this course.
9. Extra credit opportunities: There will be two articles available for you to read. Each submission can earn up to 5 points. The reference will be posted on Blackboard. You are to write a short response (1 to 2 pages) addressing questions posed on Blackboard. Paper 1 is due on or before February 9, 2007. Paper 2 is due on or before March 14. NO late submissions will be accepted. These papers will be held in a file folder. When determining final grades, if your total point value is close to the next grade, these papers will be evaluated. Submitting these papers does not mean that you will get the full point value.

Required Texts (& materials)

1. "The Power of Plagues" By Irwin Sherman
2. "The Cobra Event" By Richard Preston
3. One of the following:
   "The Demon in the Freezer" by Richard Preston or "Mountains Beyond Mountains" By Tracy Kidder
4. Lab packet – available at the Bookstore

Evaluation will be in lecture and in the laboratory according to the following schedule (tentative):

Lecture exam date	**Points**
February 12***	100
March 14	100
April 6	100
Final Exam	150

*****NOTE: this is changed from the date in the course policy in the lab manual.**

Final Exam Schedule:	9:20 Lecture	S May 5	9 – 12 N
	10:25 Lecture	W May 2	2 – 5 pm

Assignments	**Due Date**	**Points**
Proposal for Group Project	Week of Jan 29	20
Hand Washing Paper	Week of Feb 12	15
ELISA/Vaccine	Week of Feb 19	20
MIC	Week of April 9	30
Lecture Presentation	Varies	20
Reading Response	Week of April 16	30
Group Project	Week of April 23	50
Pre & post test	Week of Jan 15 & at the time of final	15

*__You MUST take Both the pre & post test to get the 15 points. The number of points you will earn will depend on your score on the post test. The pre-test must be taken the first week of the semester.__

Biology 110: Emerging Infectious Diseases (Tentative Schedule)

DATE (WEEK OF)	LABORATORY
15 Jan	Lab introduction / Discussion of final project / Pre-test
22 Jan	Laboratory Tools: Part I / Plan Hand Washing
29 Jan	Laboratory Tools: Part II, Hand Washing
5 Feb	Antigen-Antibody Interactions/Data analysis
12 Feb	Vaccination Readiness for Biological Warfare: ELISA
19 Feb	Spontaneous mutations/Bacterial Conjugation
26 Feb	MIC induction study: Begin
5 Mar	SPRING BREAK– NO LAB
12 Mar	Induction study continues: Set up tubes/data analysis(article)
19 Mar	Kirby Bauer antibiotic sensitivity study/Induction study cont
26 Mar	Post induction MIC's: Kirby-Bauer results
2 Apr	Using DNA to Screen for Smallpox/Agarose gel electrophoresis
9 Apr	HIV simulation
16 Apr	EM presentation/ HIV phylogeny
23 Apr	Group Presentations

Final Exam Schedule: 9:20 Lecture: S May 5 9:00 – 12:00 N
10:25 Lecture W May 2 2:00 – 5:00 PM

PAPER TOPICS

24 January: **O**verview of Legislative Public Policy
31 January: Uses for detection with antibodies: measles, mononucleosis
7 February: Eugenics
14 February: Use of genetically modified organisms
21 February: Nosocomial infections
23 February: Use of antibiotics in agriculture/animal husbandry
26 February: STD
28 February: Tuskegee study
16 March: Sverlodosk
21 March: Flesh eating streptococci
23 March: *E.coli* H7:0157
26 March: Methicillin and vancomycin resistant *Staphylococcus aureus*
28 March: Salad Bar Incident in Antelope, Oregon (Rajneesh)
30 March: Biowar & Bioweapons
2 April: HIV policy in Cuba
4 April: Surveillance policy for HIV
9 April: Controversial evidence: Can HIV cause AIDS?
11 April: Privacy issues for HIV
18 April: *Ebola*
20 April: Emerging infectious viruses

Project Guidelines:

1. You and a partner need to sign up for one topic for the semester. Please note the date for your presentation. You MUST present the day you are scheduled for. Failure to do so will result in forfeiture of the allocated points. NO exceptions!
2. It is your responsibility to get a topic and a date by the end of the second week of classes (26 Jan). Failure to do so will result in forfeiture of the points.
3. We expect you and your partner to work together. To that end, please communicate any difficulties you are experiencing with your partner EARLY (not the night before) in the process.
4. We will provide one reference for you to begin this project.
5. You will need at least two additional sources for this presentation (one of which needs to be a print document, not a web site).
6. This will be approximately a 10 minute presentation. You need to have visual aids (power point slides are recommended). You will only have a maximum of 15 minutes for this presentation. Plan accordingly.
7. Each pair is to submit a SHORT document outlining their presentation. This should be emailed the day prior to your presentation. Please include your references.
8. These topics are meant to be controversial. We expect you to present the topic to stimulate interest and discussion. Feel free to present both sides (pros and cons) of issues (where appropriate).
9. As an audience, we expect you to be engaged and get involved.
10. Evaluation will be based on: (1) the depth of your research, (2) the preparation of your slides, (3) your ability to communicate your information (do NOT read directly off your slides), and (4) your comfort and command of the material

Lecture Topics Resources

This is a list of the topics we will cover in lecture in order (tentative). For each topic, we have provided resources for you to use to assist you with the material.

Introduction Text: chapters: 1,2

Epidemiology Text: pages 12 -18
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap14.html

Immunology Text: chapter 10
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap15.html

Malaria Text: chapter 7

DNA
http://student.ccbcmd.edu/~gkaiser/biotutorials/dna/dna_index.html
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap8.html
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap5.html
http://carbon.cudenver.edu/~bstith/transla.MOV
http://gslc.genetics.utah.edu/units/basics/transcribe/
http://www.dnalc.org/ddnalc/resources/pcr.html
http://highered.mcgrawhill.com/sites/0072437316/student_view0/chapter14/animations.html#
http://student.ccbcmd.edu/courses/bio141/lecguide/unit6/genetics/protsyn/translation/tran slat.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit6/genetics/protsyn/translation/50s.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit6/genetics/protsyn/translation/peptidea.html

Prokaryots
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap3.html
http://www.microbelibrary.org/images/Gauthier/Endopore_formation.htm

Antibiotic Resistance
http://www.hhmi.org/biointeractive/animations/conjugation/conj_frames.htm
http://www.learner.org/channel/courses/biology/archive/animations/hires/a_infect3_h.html
http://highered.mcgraw-
hill.com/sites/0072556781/student_view0/chapter13/animation_quiz_2.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/translat.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/aglycomiscode_anim.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/tetres.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/macresp_anim.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/macresr_anim.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/macresr_anim.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/altrib.html
http://student.ccbcmd.edu/courses/bio141/lecguide/unit2/control/altpor.html
Levy, S. 1998. The Challenge of Antibiotic Resistance. Sci. Amer., Mar: 46 – 53.

The Plague: Text: Chapter 4

Syphillis Text : Chapter 12

Cholera Text: Chapter 8

Tuberculosis Text: Chapter 13

Leprosy Text: Chapter 14

Virology
http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap11.html

Smallpox Text: Chapter 12

HIV Text : Chapter 5
http://student.ccbcmd.edu/courses/bio141/lecguide/unit3/viruses/hivlc.html

Influenza
Laver, W.G., N. Bischofberger and R. Webster. 1999. Disarming Flu Viruses. Sci. Amer. Jan, 1999: 78 – 87.

Guidelines for Laboratory---Biology 110

I. Safety

1. No smoking or smokeless tobacco products.

2. No eating or drinking.

3. ABSOLUTELY NO BARE FEET. The floor may have pieces of glass and residue from spilled chemicals. (No open-toed shoes)

4. SAFETY GLASSES are may be required for certain labs. Safely glasses may be purchased at the Bookstore.

5. Read labels carefully before removing substances from a container. Never return a substance to a container.

6. Discard used chemicals and materials into appropriately labeled containers. Certain chemicals should NOT be washed down the sink; these will be indicated by your instructor.

7. Be attentive and follow instructions for lab activities, especially when helpful suggestions and safety precautions are given during the course of the laboratory period. You are responsible for your safety and other laboratory instructions.

8. Do not disturb any equipment on tables until properly instructed. If you have a problem with a microscope or other materials, please ask for assistance.

9. Know the location and use of the eye wash, emergency shower, fire extinguisher and first aid kit. In the event of a spill or splash, immediately notify the instructor or assistant.

10. You may be required at times to wear protective eyewear or gloves. Avoid exposure to gases, vapors or aerosols, especially if you have respiratory problems.

11. Wash your hands at the end of lab and clean lab tables.

12. Practice good housekeeping! Clean up your station and leave it as clean as you found it or cleaner. Remove trash on the floor around your seat. If you are missing or have broken any materials, notify the instructor.

13. No horseplay or deliberate destruction of materials will be tolerated.

II. Medical Emergency Forms: These must be returned whether you complete them or not.

MEDICAL EMERGENCY FORM

The following information may be used by qualified persons in case you are injured (e.g. by a laboratory accident) or for any other situation in which you are physically incapacitated and unable to communicate. This information will be held in strict confidence; it will be kept in an individual, sealed envelope and will be consulted only in case of emergency. The form will be returned to you at the end of the semester or destroyed.

Please print or write legibly.

STUDENT'S NAME: ____

Last First M.I.

PHYSICIAN TO BE NOTIFIED: (First priority, preferably local)

Name: ____________________________ Telephone:(___)_____________

____

(Address) (State) (Zip)

RELEVANT MEDICAL HISTORY: Answer to the best of your knowledge.

Please list all substances known to cause allergic reactions on your part.

Give any physical condition of which you are aware that might cause you to become unconscious or unable to communicate.

List any prescription medicines you take on a regular basis and the conditions for which they are prescribed.

Describe any other factors that should be considered in administering emergency treatment to you.

I authorize the use of the above information by qualified persons in appropriate situations.

Date_________________________ Signature___________________________

I choose not to present the information requested above.

Date__________________________ Signature______________________

Web Sites for Student Use

Web sites for your use:

- http://www.cdc.gov/
- http://www.cdc.gov/mmwr/
- http://wonder.cdc.gov/mmwr/mmwrmorb.asp
- http://www.cdc.gov/ncidod/eid/index.htm
- http://www.cdc.gov/epo/dphsi/phs/infdis2006.htm
- http://www.who.int/en/
- http://www.cidrap.umn.edu/index.html
- http://www.who.int/wer/2006/en/index.html

State Department of Health: Virginia or your home state

- www.vdh.state.va.us/

On-line Textbooks

- http://www.microbes.info/
- http://www.bact.wisc.edu/Microtextbook/
- http://www.lib.uiowa.edu/hardin/md/micro.html (infectious disease)

Public Health Web Sites

- http://www.apha.org/
- http://healthweb.org/publichealth/
- http://www.medicalnewstoday.com/sections/public_health/
- http://www.bt.cdc.gov/

Laboratory Group Project Guidelines

This will be a group project consisting of 4 students. The focus of this project is to have you identify an area of interest that relates emerging infectious diseases to your own chosen (or tentative) major at UR. We want you to approach infectious disease from different perspectives. In other words, clearly present the relevant biological concepts and then relate the problem to your focus.

The focus of these presentations will be an issue central to emerging infectious diseases. You will be able to ask questions and direct your research to investigate consequences of this disease. Some examples of topics that you can choose include-

- Emerging infectious diseases in war torn areas
- Biofilms
- Representation of infectious disease in the arts (movies, TV, print material)
- Prions
- Development of new drugs by pharmaceutical companies
- Vaccination strategies and controversies
- Pesticides
- History of influenza: Spanish flu, swine flu
- Other topic of your choice

In your group, you will approach this project using biological concepts as the central focus. Then, expand the research to include some (probably not all) of the following:

- Social and public policy: laws, regulations, discrimination
- Economics
- Effects on business: trade, marketing, tourism
- Any controversies surrounding the issue
- Historical perspective

There will be various components for this project. The first submission will be a project proposal as well as a division of labor in the group indicating which participant is responsible for certain information. This paper will outline the topic chosen, the interdisciplinary approach you will follow, as well as a beginning list of references. This document should be 3 – 4 pages in length and should clearly articulate the relevance of your investigation. Each person in the group MUST contribute to this document (and the contribution needs to be clearly marked). This proposal is due (in lab) the week of Jan 29.

The presentation in lab (the week of April 23) will be composed of an oral and written submission. The in-class presentation must clearly disseminate pertinent information in a clear, orderly and logical manner. However, we do not expect straight power point presentations. Here, we want you to tap into your creative energy and devise a unique addition for your talk. Feel free to include a power point component of your presentation as long as it is not the entire talk. Some suggestions include:

- Present some of your talk as a video
- Write a play, short story or a song
- Interview the microbe

- Prepare a marketing program and / or advertising campaign
- Write a news paper article
- Do a round table discussion (newsmakers)
- Use your creative abilities to devise a strategy that is compelling & engaging

Evaluation of this project will be based on

Oral presentation

- ✓ Dissemination of relevant biological material
- ✓ Scope and inclusion of the biological material
- ✓ Organization and communication
- ✓ Construction of the visual aids
- ✓ Integration of a creative feature

Written report

- ✓ Inclusion of relevant information
- ✓ Logical and organized document
- ✓ Grammar
- ✓ References

Evaluation of group members: You will be asked to complete a confidential survey evaluating the contributions of your group members.

Laboratory Tools & Skills: Pipettes & Micropipettors

Goals: As a result of this lab you will understand

- The need for different methods to deliver fluids
- How to use pipettes and micropippetors
- The difference between accuracy and precision
- How to do dilutions

Introduction

During this course we will need to utilize various tools routinely used in research laboratories. In order to successfully complete these experiments and collect meaningful data, we need to become familiar and comfortable with equipment that will be used regularly. Many of our experiments necessitate the transfer of fluids in various volumes. In order to transfer fluids we depend on pipettes.

http://www.nuncbrand.com/page/us/224.aspx#top

One type of pipettes we will be using is called serological pipettes. These pipettes will deliver volumes of fluid typically greater than 1 ml. These pipettes will be to deliver volumes of fluid up to 10 ml (but can come in even larger sizes).

http://www.indigo.com/science-supplies/gph-science-supply/pipet-pumps.jpg

There is no mouth pipetting in ANY laboratory today though it was the accepted practice not so long ago. To be able to deliver the desired volume of fluids we will use pipette pumps.

The pipette is inserted into the opening of the pipette pump and scrolling up will bring fluid into the pipette. When you are ready to dispense the fluid, scroll down or push the white lever.

Though serological pipettes are useful we will be faced with having to dispense volumes significantly smaller than 1 ml. To achieve this, we will use micropipettors. These tools can be a pleasure, making delivery of up to 1 ml easy. However, if you are not familiar with HOW they work you could easily deliver the wrong amount of liquid. So, you need to develop an intuition of what volumes "look like." These micropipettors are expensive and must be handled with care.

Some guidelines to keep in mind as you begin using these micropiettors:

- Do not dial below 0.
- Do not dial above its maximum value.
- Do not drop it.
- Do no submerge the shaft in liquid.
- Do not pound the pipettor into a tip.

http://www.vwrsp.com/catjpg/051/051376.jpg
Figure 1. Different micropipettors

In order to transfer the desired volume, tips have to be attached to the micropipettor.

There are 3 different pipettors we will be using depending on the volume we wish to dispense. The 3 pipettors we have are:

P1000 – the maximum volume is 1000 µl (1 ml). This pipettor requires the “big” tips.

P200 – the maximum volume is 200 µl (? ml) This pipettor requires the “small” tips.

P20 – the maximum volume is 20 µl (? ml) This pipettor uses the “small” tips.

www.stockwellscientific.com/ images/thumbnails...
Figure 2. Tips for micropipettors

How do these machines work? To begin, you will affix a tip to the end of the pipette shaft. Do not violently stab the tip. Press down gently and the tip will attach to the shaft. To take up liquid, depress the plunger to the FIRST stop, and then submerge the tip into the fluid. Release the plunger slowly allowing the liquid to fill the tip. To deliver the liquid, depress the plunger past the first stop to the second stop. Try this out by pipetting water or blue dye.

Exercise 1: What do different volume look like?

Demonstrate to yourself what different volumes look like. Using the appropriate micropipettor:

a. Dispense 2 µl into a small tip. Mark the tip.
b. Dispense 20 µl into a small tip. Mark the tip.
c. Dispense 200 µl into a small tip. Mark the tip.
d. Dispense 200 µl into a large tip. Mark the tip.

Look at your tips. Try and get a feel for what the volumes will look like.

Exercise 2: What does it take to mix a liquid (and why is this important)?

Your experiments (& results) are only as good as your starting materials and your procedures. Very often, experiments fail because the reagents have not been mixed sufficiently. Improper mixing puts error into your experiment. To help you gain some intuition into how important proper mixing is you will try different ways to mix dye with a glycerol-water mixture.

What you will need:
- a test tube rack
- test tubes
- 80% glycerol
- a tube of blue dye
- non-sterile microfuge tubes (small, plastic tubes)

a. Add 1 ml of 80% glycerol to a microfuge tube.

b. Add 1 ml of 80% glycerol to a test tube.

c. Add 6 ml of 80% glycerol to a test tube.

d. Add 5µl of the blue dye to the top of the 1 ml glycerol in the test tube. Try mixing the sample by moving the tube back and forth. How well do the samples mix?

e. Add 5 µl of the blue dye to the top of the 6 ml glycerol in the test tube. Try the same strategy to obtain dye-glycerol homogeneity. How successful are you?

f. Add 5 µl of the blue dye to the top of the 1 ml glycerol in the microfuge tube. Do whatever you have to do to mix the 2 liquids. What efforts are needed?

g. What conclusions have you reached about the difficulty of mixing liquids of differing viscosities and volumes?

h. Wash test tubes and invert in the test tube rack.

i. Throw microfuge tubes into waste container on your bench.

Exercise 3: **Precision vs. Accuracy - Calibration of the P1000 pipettor**

Successful completion of experiments necessitates equipment that is accurate and precise when used. In order to obtain reasonable results we have to be confident in the equipment we are using. Since we will be using our micropipettors often, we will perform an exercise to demonstrate whether or not our faith in these machines is valid. **We will utilize the relationship that 1 ml of water weighs 1 gram.**

What you will need:
- a beaker with water
- a P1000 with a tip
- weigh boat
- a balance
- your work sheets

a. Put a blue plastic weigh boat on the scale. Press “Tare” to zero the scale.

b. Using your P1000 deliver a1.0 ml aliquot of water into the blue plastic weigh boat on the scale. Record that number in columns 1 & 2 in Table 1. Remembering that 1 ml of water weighs 1 gram record the volume of the aliquot in column 3 in Table 1.

c. Deliver nine more 1.0 ml water aliquot into the blue weigh boat. Record the accumulated weight in column 2 of Table 1. How will you calculate the weight of each aliquot?

d. After you have taken all your weights fill in columns 2 & 3 in Table 1.

Aliquot #	Accumulated weight (gm)	Weight aliquot (gm)	Volume of aliquot (ml)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Table 1. Calibration of the P1000 micropipettor

In this experiment, how do we know our pipettor is accurate?

How is precision assessed?

Throughout the semester we will be using micropipettors often. It will be very helpful if you memorize the conversion where 1 ml = 1000 µl.

Exercise 4: Practice with dilutions

There will be many experiments this semester that will require you to accurately pipette and dilute a bacterial sample. In many studies, the concentration of the bacteria must be reduced or the working concentration of the bacteria has to be known. The technique used to achieve a decreased bacterial concentration is called serial dilutions. In preparation for next week we will practice the procedure and calculations necessary to be successful at serial dilutions.

What you will need:
- microfuge tubes
- water
- micropipettors & tips
- blue dye – assume the concentration of the blue dye is 10^7 particles dye / ml

<u>PREPARE THE DILUTIONS:</u>

a. Label 4 microfuge tubes A, B, C & D.

b. Pipette 900 µl of water into tubes A,B,C & D.

c. Pipette 100 µl of the blue dye into tube A. Mix well.
   How many dye particles are there in 100 µl? ____________
   What is the concentration of blue dye particles in tube A?________

d. Pipette 100 µl from tube A into tube B. Mix well.
   How many dye particles are there in the 100 µl sample?_________
   What is the concentration of blue dye particles in tube B?

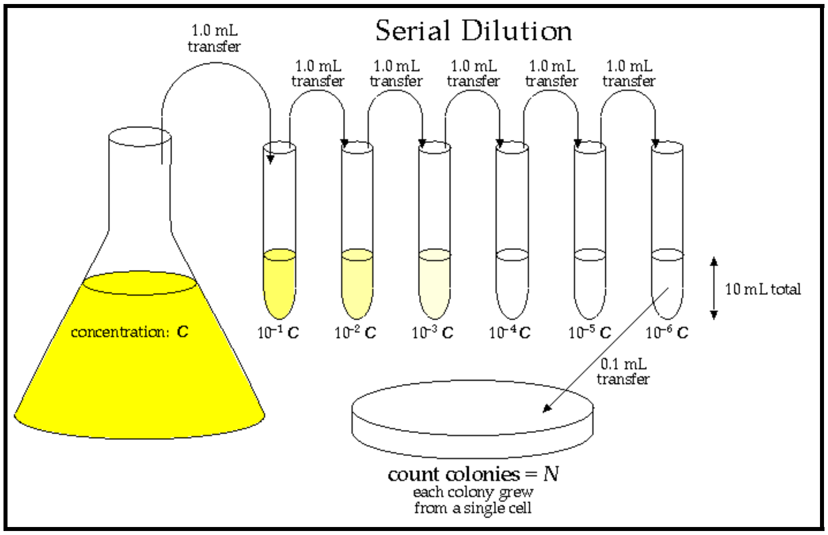
e. Pipette 100 µl from tube B into tube C Mix well.
   How many dye particles are there in the 100 µl sample?_________
   What is the concentration of blue dye particles in tube C?________

f. Pipette 100 µl from tube C into tube D. Mix well.
   How many dye particles are there in the 100 µl sample?_________
   What is the concentration of blue dye particles in tube D?________

STOP here. You will do Exercise 4: Calculating the starting volume of two strains of *E. coli* using dilutions in lab the week of January 29, 2007.

Exercise 5: Calculating the starting volume of two strains of *E. coli* using dilutions

There are many areas where you want to calculate the numbers of bacteria present in a population. You may be investigating food poisoning, beach contamination or calculating a mutation rate. In any event, bacteria can grow to very dense populations. Many species can achieve 10^9 cells per ml in less than 24 hours. Counting these cells is a daunting proposition. Instead, we rely on using a representative sample of the population to work with. To get this sample, we perform a technique called serial dilutions. This is a step-wise procedure where the sample gets "diluted" in a planned pattern.



www.physics.csbsju.edu/stats/serial_dilution.gif

Figure 3. Serial dilutions

Examine Figure 3. What relationship do you see between the concentration (the concentration resulting from dilution of the original bacterial culture) and the dilution factor (accumulated dilution from each step)?

Using dilutions we will calculate the original volumes of two strains of *E. coli*. *E. coli* is a bacterium that can be found in the environment as well as our intestines. This microbe is credited with many advances in genetic engineering, promoting health as it is a commensal microbe, causing infections and use as an indicator of contamination in natural waters.

In this exercise, we will take advantage of a nutritional requirement of *E. coli*. One strain that we will use can "eat" lactose while the other strain cannot. These two strains can be distinguished on the medium we are using based on the metabolism of lactose. Those strains that can use lactose will produce by-products that result in a pink color while the strains that cannot use lactose will appear cream. By counting the cells and using a ratio, we can estimate the original volume of each *E. coli* in the sample you will receive.

What you will need:
- 200 µl of the *E. coli* mixture (approximately 2 X 10^9 cells/ml)
- 0.9% sterile saline (for dilutions)
- MacConkey agar (4 plates per table)
- Pipettors & tips
- Colony spreaders

Alcohol lamps
Alcohol in glass plates
Microcentrifuge tubes and racks

PREPARE THE DILUTIONS:

a. Label four sterile microfuge tubes A, B, C, and D.
b. Label the agar side of the plates A, B, C and D. Include your initials and lab section.
c. Your instructor will give you a microcentrifuge tube with 200 µl of the *E. coli* mixture. Record the tube number here:__________________________.
d. **The estimated concentration** (see page 15) **of the *E. coli* is**:____________________. This number has been entered into Table 2 (1^{st} row, estimated concentration).

Prepare your dilution tubes.

e. Add 990 µl of the saline to tubes **A** & **B,** and 900 µl to tubes **C** & **D**.

Begin dilutions.

f. FIRST DILUTION. Add 10µl of the original *E. coli* mixture (in the tube you were given) to tube **A**. Mix thoroughly. The ratio of the volume of *E. coli* (10 µl bacteria plus 990 µl saline = 1000 µl) added in tube **A** to the amount of saline in tube A is the dilution factor for this step.
   The dilution factor for this dilution is:__________________________________.
   Record this number in Table (2^{nd} row, 3^{rd} column – Dilution Factor).

   The accumulated dilution factor for this dilution is the product of ALL dilution factors thus far.

   The accumulated dilution factor is:________________________________.
   Record this number in Table 2.

   The concentration of *E. coli* in the tube is the original concentration (2×10^9 cells/ml) divided by the accumulated dilution factor (10^2). Note that the units of concentration is cells/ml/

   The estimated concentration of *E. coli* in tube A is:________________
   Record this number in Table 2.

g. SECOND DILUTION. Add 10 µl from tube **A** to tube **B.** Mix thoroughly.
   The dilution factor for this dilution is:________________________.
   The accumulated dilution factor is:__________________________.
   The estimated concentration of *E. coli* in tube B is:____________.
   Record these three values in Table 2.

h. THIRD DILUTION. Add **100 µl** (note that volume of the sample has changed)from tube **B** to tube **C**. Mix thoroughly.
   The dilution factor for this dilution is:________________________.
   The accumulated dilution factor is:__________________________.
   The estimated concentration of *E. coli* in tube C is:____________.
   Record these three values in Table 2.

i. FOURTH DILUTION. Add 100 µl from tube **C** to tube **D**. Mix thoroughly.
The dilution factor for this dilution is:________________________.
The accumulated dilution factor is:__________________________.
The estimated concentration of *E. coli* in tube D is:____________.
Record these three values in Table 2.

j. SPREAD DILUTIONS ON PLATES. Pipet 100µl of the dilution in tube **A** to plate **A**, **B** to plate **B**, **C** to plate **C**, and **D** to plate **D**.

k. **Sterilize the spreader**. Before sterilizing the spreader clean off your bench of all papers, books, and personal belongings. Tie back your hair and roll up shirt sleeves. Carefully dip the metal spreader in 95% ethanol. Let the excess ethanol drip into the glass plate. Cover the plate. Using caution, place the metal spreader coated with ethanol into the flame (it will ignite). Point the spreader down so excess alcohol drips away from you. Briefly cool the spreader (hold it in the air for a few seconds). Once cooled, use the metal spreader to distribute the bacterial sample on the surface of the agar plate. After you have finished sterilize the spreader using the procedure previously used. DO not contaminate your benches with the bacteria on the spreaders.

You spread 100 µl of each sample on a plate. Remember, when we calculate dilutions (and concentrations) we always use per ml. What part of 1 ml is 100 µl?

This is called the dilution factor for plating. Record the dilution factor for plating here:__________________________.

The estimated NUMBER of cells on plate A is:______________________. We started with 2 X 10^9 cells/ml. The sample was diluted (10^2 as a dilution and 10^1 for plating)resulting in the accumulated dilution being equal to 10^3. Record it in Table 2.

The accumulated dilution factor for plate A is:_____________________________.
(Remember, accumulated dilution refers to ALL dilution steps + the dilution from plating.)

How can you count a "cell?" EVERY cell that falls on the plate will grow and divide as the plates are incubated at 37^o C. Over that time, the numbers of cells will get so great that they wall form a visible unit called a **colony**. Therefore, each colony represents where one cell landed on the agar plate.

PLATE B:
The estimated NUMBER of cells on plate B is:______________________. The original sample was diluted 10^2 in tube A , and another 10^2 in tube B. Remember to take into account the 10^1 plating factor when calculating the accumulated dilution. Record it in Table 2.

The accumulated dilution factor for plate B is:____________________________.

PLATE C:
The estimated NUMBER of cells on plate C is:_____________________. Record it in Table 2.

The accumulated dilution factor for plate C is:____________________________.

PLATE D:
The estimated NUMBER of cells on plate D is:_____________________. Record it in Table 2.

The accumulated dilution factor for plate D is:____________________________.

INCUBATE THE PLATES: in a 37^o C for 24 hours and stored until next lab.
MAKE PREDICTIONS.

Which plate(s) do you think will be so crowded with colonies that it will be uncountable?

Which plate(s) will have colonies that can be counted?

COLLECT THE DATA (next lab): For each plate, count the number of red colonies and cream-colored colonies. If the individual colonies cannot be counted because of crowding, note it here.

What is a reasonable number of colonies? Typically, we like to score plates that contain between 30 to 300 colonies. More colonies than that may be difficult to count, and less than 30 might compromise precision. These dilutions were designed to err on the side of excess, because the number of cells in the original tubes might decrease but will never increase.

	Volume of culture	Volume of diluent	Dilution factor[1]	Accumulated dilution factor[2]	Estimated concentration (cells/ml)[3]	Estimated number of plated cells
mixture	--	--	--	--	$2x10^9$	
Dilution A	10 µl stock	990 µl				
Dilution B	10 µl **A**	990 µl				
Dilution C	100 µl **B**	900 µl				
Dilution D	100 µl **C**	900 µl				

[1]Calculated as the ratio of (volume of diluent + delivered culture) to volume of delivered culture.
[2]Calculated as the product of dilution factors up to that point in the serial dilution. For plates C and D be sure to include the extra dilution realized in plating 100 µl of sample.
[3]Calculated as the expected concentration of cells in the original mixture divided by the accumulated dilution factor. Use as the estimated $2\cdot10^9$ cells/ml, since it is composed of a mixture of two stationary phase cultures)

Table 2. Dilution of mixture of *E. coli* strains

Compare your results with your predictions. How well did they agree? Discuss problems with your data.

CALCULATE THE ORIGINAL VOLUME OF *E. COLI* IN TUBE (YOUR TUBE)_____________.

Colony color	# of colonies	%[1]	Volume(µl)
red			
cream			
Total			

[1] % refers to the proportion of one colony color relative to the TOTAL number of colonies X 100.

Table 3. Proportion of red and cream colored colonies isolated

You now know the percentage of each type of *E. coli* in your original tube. The original tube contained 200 µl. What volume was contributed by the cream colored and red colored colonies? (Hint: multiply the % of each color by the total volume to get the fraction contributed by that colony color) Record that number in Table 3.

References:

*Elhai, J., B. Goodner, V. Kish, and P. Lessem. 2001 Genetics Laboratory Manual, University of Richmond
http://www.nuncbrand.com/page/us/224.aspx#top
www.stockwellscientific.com/ images/thumbnails...
http://www.vwrsp.com/catjpg/051/051376.jpg
www.stockwellscientific.com/ images/thumbnails...
www.physics.csbsju.edu/stats/serial_dilution.gif

Dilution Problems*

(Adopted from: J. Elhai and B. Goodner)

1. You have a tube of growth medium containing $2 \cdot 10^9$ cells of *E. coli* per ml.

1a. You'd like to take from this tube $2 \cdot 10^8$ cells of *E. coli*. How many milliliters must you pipet from the tube?

2. You go back to the original tube of growth medium containing $2 \cdot 10^9$ cells of *E. coli* per ml. This time you want to make a fresh tube of 1 ml of *E. coli* at a concentration of $4 \cdot 10^7$ cells/ml.

2a. How many cells of *E. coli* will be in the final 1 ml suspension of $4 \cdot 10^7$ cells/ml?

3. Fill in the blanks in the dilution scheme below, designed to achieve the desired objective:

Culture	Operation	Concentration	Cells in 100 µl (= cells on plate)
Culture **0**	(original culture)	$2 \cdot 10^9$ cells/ml	$2 \cdot 10^8$ cells
Culture **A**	990 µl medium + 10 µl of Culture **0**	$2 \cdot 10^7$ cells/ml	$2 \cdot 10^6$ cells
Culture **B**	990 µl medium + 10 µl of Culture **A**		l
Culture **C**	995 µ medium + 5 µl of Culture **B**		

4. Starting with a *E. coli* culture of $2 \cdot 10^9$ cells/ml, devise a dilution and plating scheme that would put 1000 cells on a plate. It is helpful to make a table similar to that in Problem **3**.

*Note that "µ", as in "µl" and "µg", means "micro" (10^{-6} liters or grams) and "m", as in "ml" and "mg", means "milli" (10^{-3} liters or grams).

Handwashing Paper

-15 points
-due week of October 9 (in lab)
-type, individual work, pledge
-title your lab report

1. Hypothesis
2. Results---Tables 1 and 2 (titled)
3. Discussion
    a. How did the protocol work
    b. Any weaknesses with the protocol
    c. Variables---what couldn't you control
    d. Why is handwashing important----refer to your reference
    e. Are different soaps important?
    f. Any thought about using soap with antibiotics in them?
4. References: at least one (cited)

Use of the Ouchterlony Procedure to Illustrate Antigen-Antibody Interactions

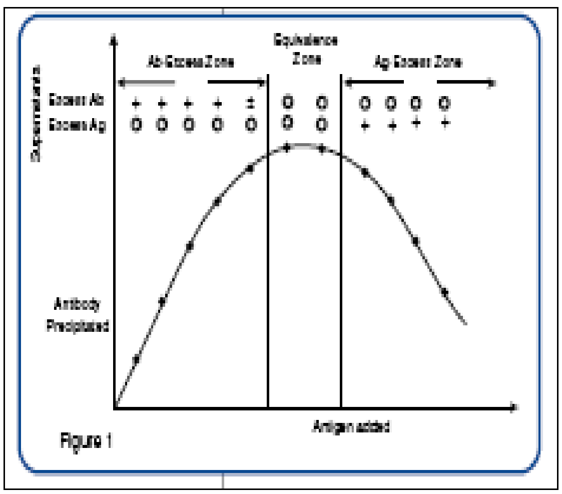
Goals:

- To understand antigen-antibody reactions by visualizing precipitation
- To be able to differentiate between zones of identity, partial identity and nonidentity

Introduction

Our body's defense, or the immune reaction, is predicated upon antigen-antibody interactions. Antigens (Ag) are protein molecules that are perceived as foreign (or not belonging) when introduced into the body. Our defenses sense and recognize this foreign material and respond. One response mechanism is the production of an antibody (Ab). The Ab will bind with the Ag forming insoluble(cannot remain dissolved) complexes that can precipitate(form a mass complex that falls out of solution). This property makes it feasible to perform quantitative and qualitative assays on this interaction.

Precipitation results from one Ab being able to bind more than one Ag. The situation is further complicated by the fact that the Ag can also bind more than one Ab resulting in the formation of a macromolecular complex. Interactions between Ag and Ab are studied by varying the amounts of each molecules.



From: .edvotek.
Fig. 1. Ag-AB interactions

To begin, the Ag is added to a constant amount of Ab where the amount of Ab (in solution) greatly exceeds the amount of Ag that has been added. This is defined the **Ab-excess zone**.

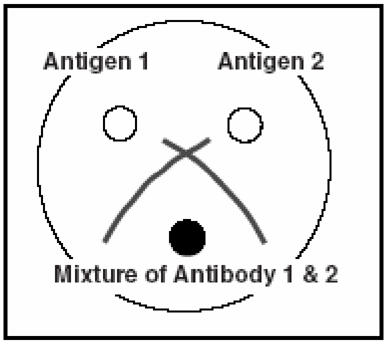
More Ag gets added until the Ab and Ag molecules are roughly in equal proportions. This is called the **equivalence zone**.

As more Ag is added to the solution, there will come a point when there is more Ag in solution than Ab and precipitation will decrease. This is the **Ag-excess** zone.

Ab and Ag can be inserted into wells in an agarose gel. Once the proteins enter the gel, they can diffuse towards each other. At the interface of their diffusion fronts, opaque bands of precipitate form. This reaction can be useful in analyzing various Ag-Ab reactions in a system.

Double diffusion occurs in two dimensions and was invented by a Swedish scientist, Ouchterlony. In this process, Ag and Ab solutions are placed in individual wells cut in an agarose plate. The reactants will diffuse towards each other and precipitin bands will form where they meet (and recognize each other) and are in equivalent proportions.

This technique can be used when comparing Ag and looking for a number of identical or cross-reacting determinants. The experiment places solution of Ag into two adjacent wells and a homologous Ab is placed in the center well.



From: edvoetk
Fig. 2. Reaction of non-identity

Reaction of non-identity: Placing unrelated Ag in adjacent wells with Ab to both Ag in the center well, the precipitin bands that form will be independent of each other and will cross. This is a reaction of non-identity.

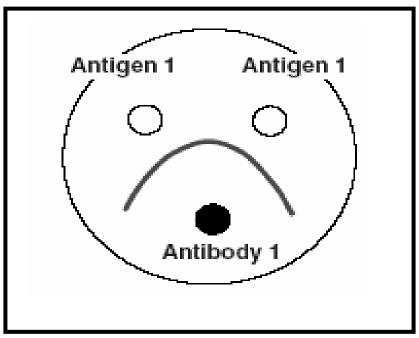


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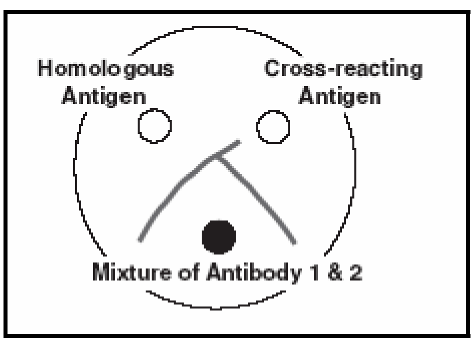
Fig. 3. Reaction of identity

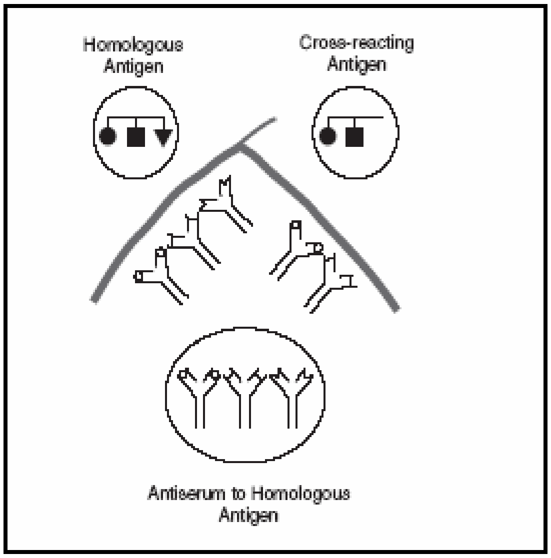
Reaction of identity: two precipitin bands that form will join at their closest end and fuse. This is known as the reaction of identity



From: edvotek.
Fig.4. Reaction of partial identity

Reaction of partial identity: You can place an Ag in one well and the Ab in another well that are a homologous pair and place an Ag that will cross react in an adjacent well results in precipitin lines that will fuse. This is known as a reaction of partial identity.

With these reactions, a spur forms that represents the reaction between the homologous Ag and the Ab that do not combine with the cross reacting Ag. The homologous Ag contains determinants that are recognized by the homologous Ab which are not present on the cross-reacting Ag. The non-cross-reacting Ab only compose a small portion of the total Ab involved in these reactions, the spur is usually less dense than the precipitin band from which it projects (Fig. 5)



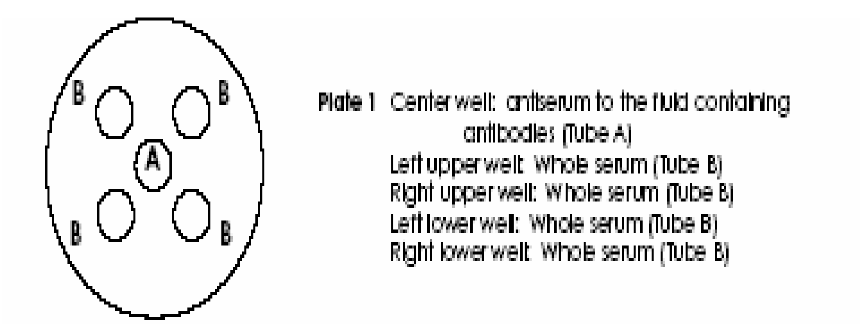
From: edvotek.
Fig.. 5. Homologous and Cross Reacting Antigens

What we will do: How do Ag and Ab react? What do precipitin bands look like?

<u>Materials</u>

Agarose plates
Micropipettors and tips
Antigens
Antibody solution

1. **Label your plates** with your initials, and "Plate 1", :Plate 2", and "Plate 3."
2. **Using the same pipette tip, fill the center wells** of all three plates with 30 µl from tube A.
3. **Fill the outer wells** following the diagrams below (Fig.)



From: edvotek

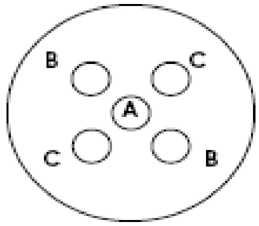


Plate 2 Center well: antiserum to the fluid containing antibodies (Tube A)
Left upper well: Whole serum (Tube B)
Right upper well: albumin (Tube C)
Left lower well: albumin (Tube C)
Right lower well: Whole serum (Tube B)

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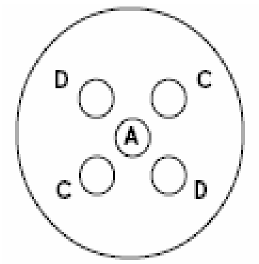

Plate 3 Center well: antiserum to the fluid containing antibodies (Tube A)
Left upper well: IgG (Tube D)
Right upper well: albumin (Tube C)
Left lower well: albumin (Tube C)
Right lower well: IgG (Tube D)

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4. **Incubate the plates**. Replace the lids on the plates and carefully place the Petri dishes on a wet paper towel in the incubator (37^{o} C).
5. **Do not invert the plates**.
6. **Collect the data**. Hold the plates up so the overhead light shines through. Look for opaque white arcs where the antibody and antigen precipitated.
7. **Draw your results**. Make a drawing of each plate. Be sure your drawings are labeled.
8. **Bring your results to lecture**.

Data Analysis

1. Explain how qualitative observations can be performed on an antigen-antibody system.

2. What is the equivalence zone or equivalence point?

3. When would you observe the antigen-excess zone? What affect does this have on the amount of precipitation?

4. What would cause two or more precipitin bands to form in an antigen-antibody experiment?

Reference: Edovtek Kit #270: Antigen-Antibody Interaction: The Ouchterlony Procedure

Vaccination Readiness for Biological Warfare/ELISA

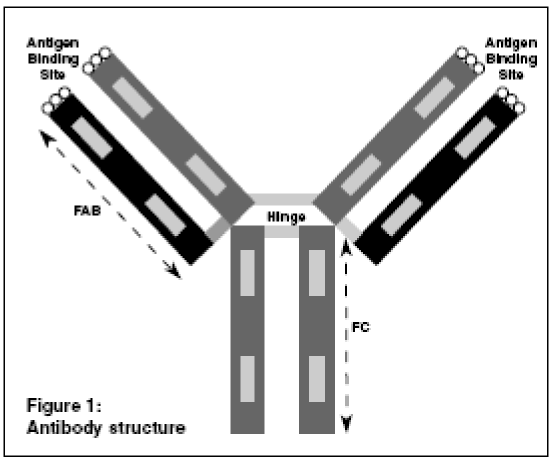
Goals:

- To investigate the different levels of immunity among participants
- To understand what an ELISA is

Introduction

Immunity is defined as the ability of the body to resist invasion by foreign species termed antigens (Ag). These foreign materials can be bacteria, fungi, viruses and tumor cells. The human response is mediated by two different mechanisms: (1) a cell-mediated response consists of actions taken by T cells while (2) the humoral response depends on the actions of B cells. Though there are two distinct groups of cells, their ability to fight off foreign species is interrelated and these two branches are not separate from each other.

The process is initiated when the body perceives an object as foreign (Ag) which stimulates the B cells to produce antibodies (Ab) specific for that particular Ag.



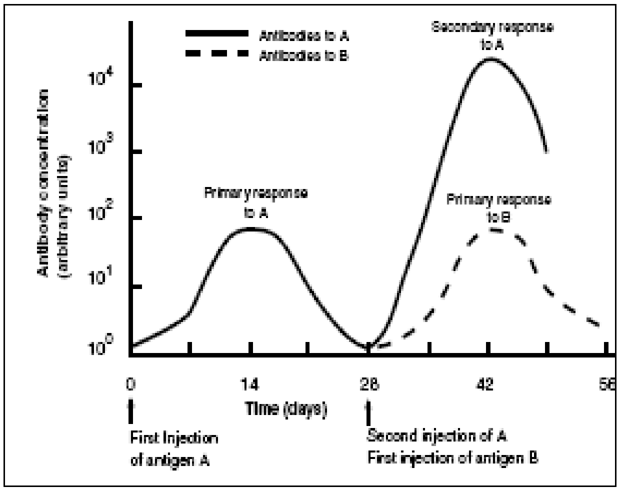
From: Edvotek
Fig. 1. Antibody structure

Structure of an antibody:
F_c = the constant region

F_{ab} = the variable region for antigen binding

Ab come in different classes: IgG responds to bacterial and viral infections

What happens when the immune system gets stimulated? Once an Ag is perceived, a subset of the B cells is stimulated to produce Ab against this Ag and does so for a long period of time. These primary responders have an additional function - they serve as memory cells. An additional exposure to the same Ag will stimulate a secondary response in a much greater magnitude than the primary response. In Fig. 2 the reaction to two different Ag, A and B, is illustrated. This figure illustrates the primary immune response to Ag A when compared to a subsequent exposure to the same Ag. The secondary response clearly shows a much greater titer (concentration of circulating Ab). Concurrently with the second exposure to A, this person is also exposed to Ag B. Based on the figure, this was the “first” encounter with this Ag resulting in a primary response based on the antibody titer.



From: Edvotek
Fig.2. Ab response to Ag

The ability of the immune system to produce memory cells is the basis for vaccination. The purpose of vaccination is to generate a population of B cells that have memory to a particular Ag prior to actual exposure to that agent. The process entails administering bacterial or viral Ag to a person resulting in the stimulation of competent B cells. However, this population of B cells is not static and diminishes over time. Therefore, booster shots are necessary to ensure immunity.

The preparation of vaccines can have varied approaches. There are pros and cons to each of the different preparations. The most basic line of attack is to use killed bacteria or viruses. Obviously, an advantage of this approach is that there is no way that the person can come down with an active infection caused by this agent. One negative aspect of this approach is that without replication there is less Ag presented resulting in a diminished immune response. In addition, the manufacturer must ensure that all the organisms are killed and that there is no toxin in the preparation.

Another tactic is to use a live (a related strain) or an attenuated (less virulent) strain of the infectious agent. One example where living microbes are used is smallpox. In this case, the vaccinia virus is used which is related to the variola virus (smallpox) but it less virulent. The main advantage of this approach is that the immune response is greater because there are living organisms replicating, increasing the viral load, resulting in a stronger primary response. One major disadvantage of this system is that the disease causing microbe may be sufficiently different from the microbe in the vaccine rendering it ineffective. Additionally, when live viral particles are being there is the possibility of an actual infection occurring and there is a chance of side effects.

A third strategy is to use one part of the infectious agent (a toxin or a surface Ag) that is present in high enough concentrations to be able to stimulate a primary response which would be efficient in conferring immunity when infected with the whole microbe. Various vaccines use this approach including the Hepatitis B virus where the surface Ag is used in the vaccine. Tetanus and diphtheria use formaldehyde treated exotoxins to mount a primary response. The obvious advantage to this approach is that no live microbes are introduced resulting in no negative health impact. In this approach, the toxin must be completely inactivated before administration.

Future approaches to vaccination include the use of naked DNA (or RNA) attached to microscopic gold beads. This DNA (or RNA) is responsible for encoding Ag proteins and is injected into muscle tissue. In the tissue, the genes are expressed and the protein is secreted which results in stimulating the production of B and T cells. The advantage of this system is that it is low cost with no chance of infection. One disadvantage is the there is a possibility of triggering an autoimmune reaction.

There are various serological (looking for molecules in the serum) methods that are employed to determine if a person is "immune" to a certain infectious agent. In order to demonstrate immunity, there needs to be a specific Ab in the serum. The method that we are going to do is called ELISA – Enzyme Linked ImmunoSorbent Assay. This assay uses a microtiter tray (similar to what we have previously used), antibodies and an enzyme-substrate reaction. The Ag that you are screening for is bound to the polycarbonate microtiter plate. Serum is then added to the well. If the serum contains Ab to the Ag attached to the polycarbonate plate, the Ab will bind with the Ag (remember Ag-Ab interactions are highly specific). Once the Ab has bound to Ag the entire complex is bound to the well and will not be removed by washing (Fig. 3).

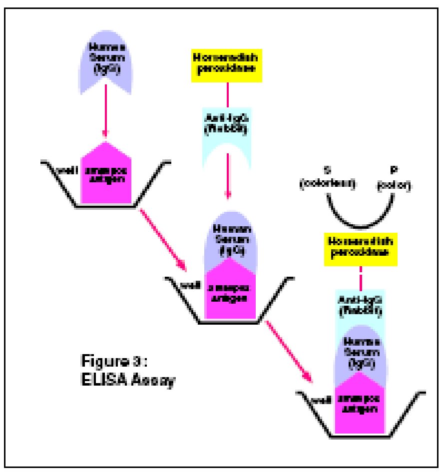


Figure 3: ELISA Assay

From: Edvotek

So, how are we able to detect this interaction? Both components are clear and colorless. What we must do is add more chemicals that will ultimately result in a color change if our Ab is present in the sample. After we have washed the wells (to remove any unbound Ab), we add another Ab (called a secondary Ab because it is not specific for the Ag but will bind with the constant part of the Ab molecule) that is conjugated (attached to) an enzyme. If the Ab-Ag complex is present, the secondary Ab will bind and is now attached to the plate. If the Ab (specific for the Ag) is not present, NO Ab will have bound to the Ag so there will be no molecule for the secondary Ab to bind with. The wells are washed again.

How do we complete this assay? We basically have two alternatives:

(1) the serum contained the Ab and is attached to the Ag. The secondary Ab has bound to the Ag-Ab complex and is attached to an enzyme.

(2) the serum did not contain the Ab so nothing has attached to the Ag. The secondary Ab is washed away in the rinses. All that remains on the plate is the Ag. There is no secondary Ab nor any enzyme.

At this point we can add the substrate. A substrate is a molecule that an enzyme will react with. Enzyme-substrate interactions are very specific and are determined by the shapes of the various reactants. Just as Ag-Ab reactions are specific this is another example where the interaction is defined. The substrate is added. If the enzyme is present the enzyme will react with the substrate and the product is colored. Any color development indicates substrate cleavage which means that the secondary Ab (with the enzyme) is present.

What we will do: We will use the ELISA method to investigate the immunity status of different people in a community. The population that we will test includes individuals that are unimmunized, distantly immunized, recently immunized or have received a booster.

Materials

Microtiter plate
Micropipettes and tips: We will use our micropipettors and tips for this experiment.
Phosphate Buffered Saline (PBS)
Blocking agent
Patient's serum
Secondary antibody
Substrate

GENERAL INSTRUCTIONS AND PROCEDURES

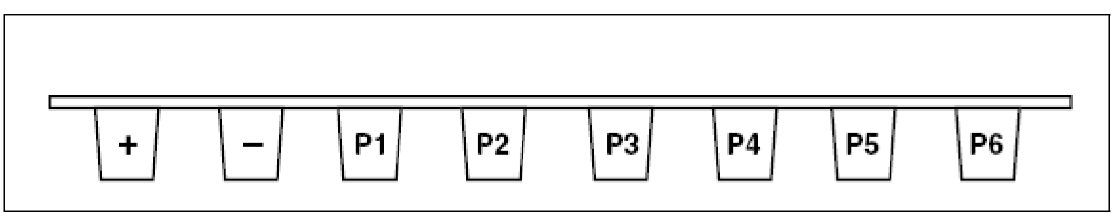
Labeling the Microtiter Plate:

1. Place the microtiter plate as shown in Figure 4.
2. Carefully mark the plate with your initials or lab group number.
3. Along the side of the microtiter plate, label the wells in the following manner (as shown in Figure 4):
   +, –, P1, P2, P3, P4, P5, and P6

Labeling the Plastic Transfer pipets:

4. Label 2 large transfer pipets as follows (these are to be used for addition of reagents to the wells):
   - PBS for Phosphate Buffered Saline
   - Block for blocking agent

CAUTION: Use the appropriately labelled plastic transfer pipet for liquid removals and washes as outlined in the experimental procedures to avoid cross-contamination and false results.



From: edvotek

Fig. 4 Model of the microtiter plate

Addition of Blocking Agent

5. Using a fresh pipet tip, add 50 µl or 1 drop of blocking agent to each of the 6 wells and incubate for 10 minutes at 37°C.

6. Use the same pipet to remove the liquid from the wells.

PBS Wash and Liquid Removal of Blocking Agent

7. Using the large pipet labelled "PBS" add Phosphate Buffered Saline to all the wells. Fill until each well is almost full. If using a micropipet, add 200µl of PBS to each of the wells.

8. Remove the PBS from the wells using a new pipet. Discard the small pipets when finished.

ADDITION OF PATIENT SAMPLES:

1. Label 6 large transfer pipets in the following manner. +, –, P1, P2, P3, P4, P5, and P6 .

2. Using a new pipet tip or clean pipet for each sample, add 50µl of each of the six samples (+ control, – control, Patient #1, Patient #2, Patient #3, Patient #4, Patient #5, and Patient #6).

3. Incubate the wells at 37°C for 30 minutes.

Liquid Removal of Control and Patient Samples

4. Remove the Control and Patient samples from the wells by using the correctly labeled pipets. Save the pipets for the next wash.

PBS Wash and Liquid Removal of PBS

5. Using the large pipet labelled "PBS" add Phosphate Buffered Saline to all the wells. Fill until each well is almost full. If using a micropipet, add 200 µl to each of the wells.

6. Remove the PBS from the wells by using the correctly labelled pipets. Save transfer pipets for the next wash.

Addition of Secondary Antibody

7. Use a fresh pipet tip or a transfer pipet to add 50 µl or 1 drop of Secondary Antibody to each of the wells.

8. Incubate the plate at 37°C for 15 minutes.

Liquid Removal of Secondary Antibodies:

9. Remove the secondary antibodies from the wells by using the correctly labeled pipets. Save the pipets for the PBS wash.

PBS Wash and Liquid Removal of PBS

10. Using the large pipet labelled "PBS" add Phosphate Buffered Saline to all the wells. Fill until each well is almost full. If using a micropipet, add 200 µl to each of the wells.

11. Remove the PBS from the wells using the correctly labelled pipets.

Addition of Substrate

12. Use a fresh pipet tip or a fresh large transfer pipet to add 50 µl or 1 drop of Substrate to the wells.

13. Incubate at room temperature for 5 minutes.

14. Periodically observe the plate for color development.

15. If color is not fully developed after 5 minutes, incubate for a longer period of time.

16. Using a fresh **small** transfer pipet, add 20 µl or 1 drop of "stop" solution to each well to stop the reaction when you are satisfied with the contrast between the different wells.

Data analysis:

1. Why are two antibodies needed in this experiment?
2. How prepared are we for a smallpox outbreak?
3. Go the following website: http://www.bt.cdc.gov/agent/smallpox/training/clinician-know/index.asp . Review the materials and information available.

References: Edvotek kit # 280

Spontaneous mutations in *E. coli*

Goals:

- To understand the use of dilution plating to answer scientific inquiries
- To evaluate spontaneous mutations as a means for bacteria to develop antibiotic resistance
- To investigate the potential of different *E. coli* strains to develop antibiotic resistance

Introduction

DNA, the genetic blueprint, is susceptible to change. These changes can have a positive or negative effect on the bacterium. However, any change to the DNA is permanent and will be passed on to future generations. Some of these changes may have a positive outcome for the bacterium (more tools for survival) and be deleterious for us. For example, mutations that result in generating an antibiotic resistant bacterium is an example where the bacterium benefits from such a change while effective antibiotic therapy is negatively impacted.

Mutations make a permanent change in the DNA. The change (in the nucleotide sequence) can be very small (a point mutation where one nucleotide is changed) to extremely large where entire sections of the DNA have been deleted.

Based on what you already know, what are the three outcomes of a point mutation:

1.
2
3.

Which of the three do you think has the greatest probability in changing the DNA such that you select for the microbe with a “new” trait (i.e. antibiotic resistance)?

How does a bacterium “get” a mutation? There are two ways that bacteria can be potentially mutated. One type of mutation is called a spontaneous mutation. NO outside source is responsible for the change in the DNA. Rather, it is an internal problem.

Suggest two ways that spontaneous mutations can arise in bacteria.

1.
2.

In addition to spontaneous mutations, changes in DNA can be induced. Many environmental factors are responsible for changes in the DNA code.

Suggest two factors that are known to induce mutations in DNA.

1.
2.

What we will do: How will we determine if there are low levels of nalidixic acid resistant bacteria in a larger bacterial population? Do all strains of *E. coli* have the same level of nalidixic acid resistance?

Nalidixic acid is an antibiotic used to treat urinary tract infections caused by Gram negative rods particularly *E. coli*, *Klebsiella*, and *Enterobacter* species. It can be bacteriostatic (stopping the growth of the bacteria) or bacteriocidal (killing the bacteria). This drug interferes with DNA synthesis.

Determine whether there are low levels of nalidixic acid resistant bacteria in an *E. coli* population. Each pair will do one strain. Decide at your table which strain you are using. Write it down here:____.

<u>Materials</u>

E. coli strains HB101 and MG6155
Agar plates with 0.04 mg/ml nalidixic acid.
Microcentrifuge tubes
Metal spreaders
Alcohol lamps
Alcohol in glass dishes

1. **Pipette 1 ml** of the culture you selected into 5 microfuge tubes. Which pipettor should you use?_____________________
2. **Spin these tubes down for 1 minute** in a microfuge (your instructor will show it to you).
3. **Label 5 agar plates** with your initials, *E. coli* strain, and lab section.
4. **Decant the supernatant**. You can either spill out the contents into your waste beaker or you can use your pipette and remove the fluid with a tip. You will have removed the liquid contents of the tube and all that is left is a solid mass on the bottom of the tube. That is the pellet.
5. **Resuspend the pellet** by adding 200 µl of sterile saline. MIX well.
6. **Pipette the entire** volume (all 200 µl) onto an agar plate that contains 0.04 mg/ml nalidixic acid.
7. **Spread the mixture** onto the surface of the agar plate using a sterile spreader.
8. **Sterilize the spreader**. Clean off your lab bench of all extra materials. Tie back your hair and roll up all shirt sleeves.
9. **Carefully dip the metal spreader in 95% ethanol**. Let the excess ethanol drip into the glass plate. Cover the plate. Using caution, place the metal spreader coated with ethanol into the flame (it will ignite). Point the spreader down so excess alcohol drips away from you. Briefly cool the spreader (hold it in the air for a few seconds). Once cooled, use the metal spreader to distribute the bacterial sample on the surface of the agar plate. After you have finished sterilize the spreader using the procedure previously used. DO not contaminate your benches with the bacteria on the spreaders.
10. **Repeat this procedure** for all 5 microfuge tubes. You will have 5 plates.
11. **Incubate** the plates at 37^{o} C overnight. The plates will be refrigerated until the next lab period.

PREDICTIONS:
Do you expect there to be any growth on the agar plates with nalidixic acid?

Are there any controls included in this protocol?

Do you expect both strains to exhibit the same number of nalidixic acid resistant cells?

How can we determine the number of bacteria in the population that are resistant and sensitive to nalidixic acid?

These two *E. coli* strains are overnight cultures with approximately 2 X 10^9 cells / ml. If you were to plate 100 µl of this culture directly onto an agar plate how many colonies would you have?__________________________________

So, to alleviate this problem we will perform serial dilutions.

1. **Label 6** sterile microfuge tubes 1 – 6.
2. **Dispense** 900 µl of sterile saline into each tube.
3. **Add 100 µl of the *E. coli*** that you are working with into tube 1. Mix well.
4. **Transfer 100 µl** of the solution in tube 1 to tube 2. Mix well.
5. **Transfer 100 µl** of the solution in tube 2 to tube 3. Mix well
6. **Continue the serial dilutions** through tube 6.
7. **Label 4 agar plates** with your initials, *E. coli* strain, lab section and dilution number (to represent the samples in 3, 4, 5 and 6).

   Why are we plating so many dilutions?

   Which agar plates (those with nalidixic agar and those without the antibiotic) are you going to use? Why did you make that choice?

8. **Spread 100 µl** of each sample (from tubes 3 -6) onto the appropriate plate.
9. **Use a sterile spreader** to distribute the cells on the surface of the agar plate. Carefully dip the metal spreader in 95% ethanol. Let the excess ethanol drip into the glass plate. Cover the plate. Using caution, place the metal spreader coated with ethanol into the flame (it will ignite). Point the spreader down so excess alcohol drips away from you. Briefly cool the spreader (hold it in the air for a few seconds). Once cooled, use the metal spreader to distribute the bacterial sample on the surface of the agar plate. After you have finished sterilize the spreader using the procedure previously used. DO not contaminate your benches with the bacteria on the spreaders.
10. **Incubate the plates** at 37^o C overnight. The plates will be refrigerated until the next lab period.

COLLECT THE DATA.

1. Determine the number of nalidixic acid resistant bacteria. Count the number of nalidixic resistant colonies on the 5 plates.

Record that data in Table 1.
Record the data from the other strain.

Plate	# colonies	
	HB101	MG6155
1		
2		
3		
4		
5		
Average		

Table 1. Nalidixic acid resistant *E. coli*

Do your results match your predictions?

Do you think the nalidixic acid resistant cells are a result of a spontaneous mutation or an induced mutation? Explain.

What difference would you expect to see at the DNA level?

CALCULATE the number of nalidixic acid per ml:

nalidixic acid bacteria per plate = # nalidixic acid resistant bacteria per ml
0.2 ml per plate

CALCULATE the number of total bacteria per ml:

bacteria X accumulated dilution (including the plating factor)=

CALCULATE the mutation frequency:

nalidixic acid bacteria per ml
total # bacteria per ml

References:
Klug, W.S. and M.R. Cummings. Concepts of Genetics, 7th Ed. Pearson Education, Upper Saddle River, NJ.
Thanks to Dr. Laura Runyen-Janecky - adopted from Genetics 2005

Bacterial Conjugation: Exchange of DNA due to Cell-to-Cell Contact

Goals:

- To utilize dilutions
- To investigate gene transfer between two *E. coli* strains
- To examine whether gene transfer can result in a more resistant *E. coli*
- To investigate the effects of various environmental conditions on conjugation

Introduction

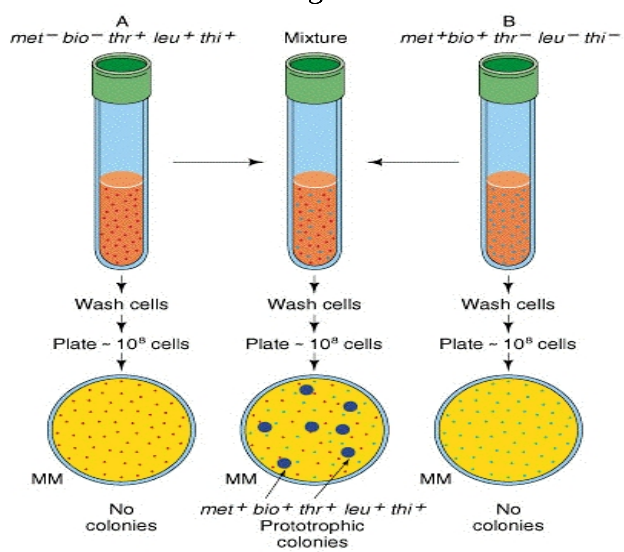
Bacteria are able to exchange DNA rather easily under various conditions. This transfer of genetic information can be accomplished through three distinct processes. All these processes are the same in that DNA from one organism is transferred to another. Some of these mechanisms are more specific where the bacterial strains have to closely related while other mechanisms afford DNA transfer between disparate genera.

The three types of DNA exchange are:

1. transformation – uptake of naked DNA
2. transduction – transfer of DNA mediated by a bacterial virus (bacteriophage)
3. conjugation – DNA exchange due to cell-to-cell contact

Conjugation was first described by Joshua Lederberg and Edward Tatum in 1946. They were working with two strains of *E. coli* that had slightly different nutritional requirements. One strain, A, requires methionine and biotin for growth while the other strain, B, requires leucine and thiamine for growth. Neither one of these strains is able to grow on minimal medium (a growth medium that does not include any amino acids).

Lederberg and Tatum mixed the two *E. coli* strains, A and B. After incubation they plated all three samples (A, B, and A and B) on minimal medium. The sample that contained both strains resulted in the growth of a few colonies (See Figure 1).



Looking at Figure 1, can you identify any controls?

Are controls important?

How can you explain the appearance of colonies on the minimal medium from the mixture of strains A and B?

www.mun.ca/.../3107/ images/Griffiths/G10-4.jpg

Figure 1. Conjugation in bacteria

It was determined that a factor, called the F (fertility) factor was responsible for the exchange of DNA. The F factor is a plasmid and contains all the information it needs to be able to transfer itself to another cell. Besides these genes it can also transfer other genes as it enters the recipient cell.

We will investigate whether antibiotic resistance can be transferred by conjugation.

What we will do: Can we demonstrate whether antibiotic resistance is being transferred from one cell to another?

Materials:

E. coli strains 1 (SY237) and 2 (SM10/pHM5)

E. coli SY237 is resistant to nalidixic acid and sensitive to ampicllin* **(A)**

E. coli strain SM10/pHM5 is resistant to ampicillin and sensitive to nalidixic acid***(B)**

Agar plates: Four different types

1. no antibiotics added
2. 0.04 mg/ml nalidixic acid
3. 0.25 mg/ml ampicillin
4. 0.04 mg/ml nalidixic acid and 0.25 mg/ml ampicillin

*Ampicillin is bacteriocidal by impeding the crosslinking in the peptidoglycan in the cell wall of bacteria.

* Nalidixic acid effects the DNA gyrase.

5. colony spreaders
6. alcohol lamps
7. alcohol glass jars

What experiment can you design to determine if antibiotic resistance genes are being transferred from one bacterium to another?

What controls would you need? What information do they provide for you?

We know that bacteria can exchange genes in a laboratory setting so our challenge is to try different conditions and see if conjugation will still occur. Compare the laboratory setting with a more natural setting. When you put two bacterial strains together what parameters can be investigated (do any of these parameters effect the transfer of DNA)?

a. time together
b. incubation temperature
c. concentration of each strain

Each pair needs to decide what factor they want to investigate and design a plan. Before you continue share your plan with your instructor or teaching assistant.

Once you have designed your plan proceed to the procedure.

1. **Pipette 1 ml** of each culture into a separate labeled microfuge tube.
2. **Centrifuge** the tubes in a microcentrifuge (top speed) for 1 minute and discard the supernatant.
3. **Resuspend** the pellet in 1 ml of sterile saline. Mix well.
4. **Add 200 µl** of strain A and 200 µl strain B to a sterile labeled microfuge tube (label conjugation tube).
5. **Incubate all three tubes (**A, B and conjugation tube) at the temperature and time period you selected. Do not move the tubes. Be sure you have recorded the time and temperature.

PREPARE THE MIXTURES FOR PLATING: These samples need to be diluted to afford accuracy and precision in data collection. All groups should use the following procedure EXCEPT groups who manipulated strain concentration as their variable. Those groups should talk with their instructor prior to beginning the dilutions.

1. **Label three sterile** microfuge tubes 1, 2, and 3.
2. **Dispense** 900 µl of sterile saline into each tube.
3. **Pipette** 100 µl of the sample from the conjugation tube and place it in tube 1. Mix well. What type of dilution is this?
4. **Pipette** 100 µl of the solution in tube 1 and place it in tube 2.
   Is the concentration of the bacteria getting higher, lower or remaining the same? Why?
5. **Pipette** 100 µl of the solution in tube 2 and place it in tube 2.
   What is the accumulated dilution for these steps?

PLATING THE SAMPLES:

1. **You are going to plate** the undiluted sample as well as dilutions 1, 2, and 3.
   There are 3 different agar plates for you to use. Which one(s) will you use? Why did you pick these?
2. **Label the agar** side of the plates containing 0.04 mg/ml nalidixic acid and 0.25 mg/ml ampicillin (if this was not your choice please reconsider). The label should contain your initials, sample description (undiluted, 1, 2, or 3) and your lab section.
3. **Dispense** 100 µl of the undiluted sample onto the appropriately labeled plate.
4. **Spread** the bacteria over the surface of the agar using a sterile spreader.
5. **Sterilize the spreader.** Clean off your benches of extra papers. Be sure your hair is tied back and shirt sleeves are rolled up. Dip the metal spreader into the ethanol, allow excess alcohol to drip into the glass dish, carefully ignite in the flame, and dip it down. Allow it to cool and use the spreader to distribute the bacteria over the surface of the agar plate. Re-sterilize the spreader and spread the other samples.
6. **Repeat** these steps for dilutions 1, 2, and 3.

CONTROLS: You need to validate your results by verifying the antibiotic resistance spectrum of your two *E. coli* strains.
Would the results be different if one strain was resistant to nalidixic acid and ampicillin?

Would the results be affected if one strain was resistant to ampicillin and the other strain was sensitive to both nalidixic acid and ampicillin?
How can we verify that *E. coli* SY237 is resistant to nalidixic acid and sensitive to ampicllin

1. **Label the agar side** of a plate containing 0.04 mg/ml nalidixic acid and 0.25 mg/ml ampicillin with the strain number, your initials and your lab section.
2. **Label the agar side** of a plate containing 0.04 mg/ml naldixic acid with the strain number, your initials and your lab section
3. **Pipette 100 µl** of the sample onto each plate (plates labeled in steps 1 & 2).
4. **Distribute** the cells evenly over the surface of the agar. Be sure to use a clean spreader (use the proper technique for sterilizing) for each plate.

How can we verify that *E. coli* strain SM10/pHM5 is resistant to ampicillin and sensitive to nalidixic acid

1. **Label the agar side** of a plate containing 0.04 mg/ml nalidixic acid and 0.25 mg/ml ampicillin with the strain number, your initials and your lab section.
2. **Label the agar side** of a plate containing 0.25 mg/ml ampicillin with the strain number, your initials and your lab section
3. **Pipette 100 µl** of the sample onto each plate (plates labeled in steps 1 & 2).
4. **Distribute** the cells evenly over the surface of the agar. Be sure to use a clean spreader(use the proper technique for sterilizing) for each plate.

Incubate all the plates at 37^{o} C overnight.

PREDICTIONS: Based on your knowledge fill in the following table BEFORE you collect your data. Indicate growth with "+" and no growth as "-".

Strains	No antibiotics	0.04 mg/ml Nalidixic acid	0.25 mg/ml Ampicillin	Nalidixic acid + Ampicillin
A				
B				
A + B				

COLLECT THE DATA:

1. **Count the colonies** on the plates. If there are over 400 colonies record the result as "too numerous to count." For all other plates, count the colonies and record them in a table in your notebook.
2. **Calculate** the number of transconjugants:

$$\frac{\text{\# of ampicillin \& nalidixic acid resistant bacteria}}{\text{dilution (0.1 ml)**}} = \text{transconjugants / ml}$$

** 0.1 ml refers to the plating factor

References:
www.mun.ca/.../3107/ images/Griffiths/G10-4.jpg
Adopted from: genetics 2005 (thanks to Dr. R-J)

Minimal Inhibitory Concentration: Induction of antibiotic resistance in bacteria

Goals:

- To investigate the potential for the development of antibiotic resistance
- To understand how to perform the Minimal Inhibitory Concentration (MIC)
- To explore the relationship between antibiotics used in agriculture as a possible mechanism to induce antibiotic resistance to drugs commonly prescribed
- To perform an independent research project

Introduction

Bacterial infections proved fatal to many people afflicted during the 1940's and earlier. With the advent of antibiotics, many of these illnesses could be cured. The medical community and patients welcomed these "miracle drugs." However, over the years, antibiotics were misused. They have been (and still are) prescribed for viral infections and have been used indiscriminately (in the not so distant past) in agriculture. The long-term effect of these misuses is the propagation of many bacterial strains that are resistant to many drugs. Today, the picture is not so rosy. Many patients have to use more than one antibiotic to combat an infection due to bacterial resistance. Additionally, there are bacterial strains that are only sensitive (sensitive implies that the bacteria will be killed by the antibiotic) to a small handful of antibiotics, some of which have uncomfortable side effects.

Antibiotics are routinely used in agriculture for many different purposes. The most accepted purpose of dispensing these drugs is to treat sick animals. However, their use is much more widespread. Animals are given antibiotics simply to prevent infection (prophylaxis) resulting in large numbers of animals being dosed. Besides preventing infection, some antibiotics seem to work as growth promoters. The animals may not grow larger but they gain weight more rapidly (Salyers and Whitt, 2005). Though the relationship between antibiotics and increase in growth has been established, the exact mechanism on how this occurs is elusive.

One antibiotic used frequently in agriculture is tylosin. Tylosin belongs to the macrolide family of antibiotics. These antibiotics work by binding to the large subunit of the bacterial ribosome inhibiting the elongation of bacterial proteins. Another macrolide, erythromycin, is commonly used to treat many different bacterial infections including wound and respiratory infections. Erythromycin is a good alternative for treatment for those that are allergic to penicillin. Yet another macrolide, azithromycin (Zithromax), is used routinely to treat various infections. These drugs are effective and present few side effects.

Tylosin is used to treat a wide variety of animals. It is used in the treatment of disease in swine, cattle, and poultry and is used as a growth promoter in pigs. While no studies have been conducted, it has been found to be useful in treating colitis in pets (http://www.greatvistachemicals.com/industrial_and_specialty_chemicals/tylosin.html).

Therefore, it appears that this drug has widespread applications in veterinary medicine as well as agriculture.

We have investigated two mechanisms responsible for the development of antibiotic resistance in bacteria. Since bacteria can exchange DNA, there is the potential for "sharing of DNA" in the guts of these animals. The waste materials of these animals could potentially contain thousands of tylosin resistant microbes that are just washed into the environment. Again, here are additional opportunities for exchange of DNA with otherwise sensitive microbes.

So, why do we care? Because tylosin is closely related to erythromycin, there is the very real potential that tylosin resistant microbes will also be resistant to erythromycin. This situation can impact health care as macrolides (especially erythromycin) are prescribed often.

What we will do: Can exposure to sub-MIC levels of tylosin result in induction (increased resistance) to tylosin? After exposure to tylosin will the bacteria become more resistant to other macrolides (Azithromycin, Roxithromycin, Erythromycin)?

Before we can investigate the effect tylosin might have on antibiotic resistance (to macrolides) we need to establish base-line values to be used as a comparison. Think of this first study as a control. Once we define its basic resistance / sensitivity pattern (using designated antibiotics) of a selected bacterial isolate we can begin the induction study.

So, what is the first thing you would suggest we do?

Materials

- Overnight bacterial culture: various bacterial strains
- Sterile nutrient broth
- Microfuge tubes
- Micropipettors and tips
- Microtiter plate
- Sterile test tubes
- Antibiotic solutions

Determining the base-line MIC to tylosin, erythromycin, azithromycin and roxithromycin: Perform MIC's

1. **Prior to the lab** (24 hours) aseptically inoculate (into a tube with nutrient broth) the bacteria you will be testing. Incubate at 37^{o}C with shaking. This was done for you.

2. **At the class meeting** the cultures need to be diluted so the MIC's can be run.
Why do you think dilutions are necessary?

The overnight culture of bacteria has approximately 10^9 cells/ml. The target concentration needed to perform MIC's is approximately 10^3 colonies/ml. Use the following dilution scheme to prepare your samples.

Sample	Volume Nutrient Broth (µl)	Volume Sample (µl)	Tube label	Concentration of bacteria
Original	990	10	A	10,000,000 (10^7)
A	990	10	B	100,000 (10^5)
B	900	100	C	10,000 (10^4)
C	900	100	D	
D*	7200	100		

Table 1. Dilution scheme to prepare samples for MICs.

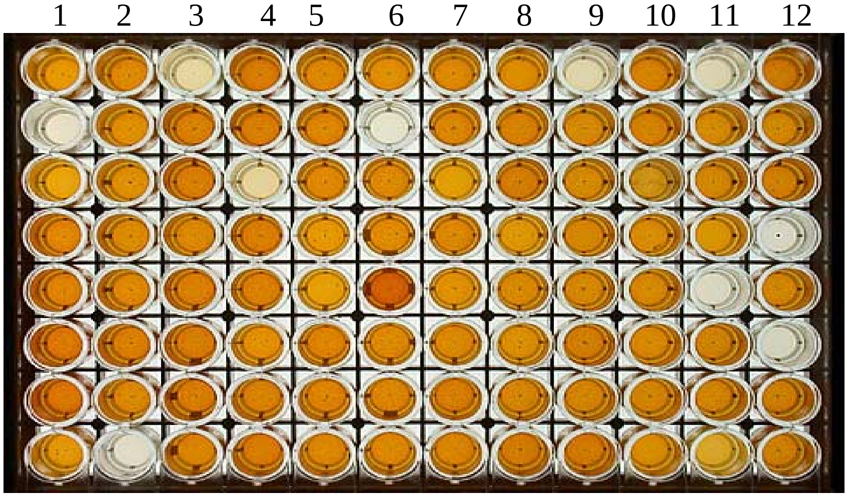
*Tube D is a large sterile test tube.

3. **Label** 3 sterile microfuge tubes A, B, and C.
4. **Label** one sterile, capped test tube D.
5. **Dispense** 990 µl sterile nutrient broth into tubes A and B.
6. **Dispense** 900 µl of sterile nutrient broth into tube C.
7. **Dispense** 7.2 ml of sterile nutrient broth into test tube D.
8. **Follow the dilution scheme** in Table 1. Pipette 10 µl of the original sample and dispense it into tube A. Mix well.
9. **Pipette 10 µl from tube A** and dispense into tube B.
10. **Pipette 100 µl from tube B** and dispense into tube C. **Fill in the last column of the table.**
11. **Dispense** 800 µl of tube C into test tube D**. Fill in the last column of the table.** Test tube D is your seeded culture medium that contains the concentration of the bacteria that we want for running an MIC.
12. Why was it necessary to dilute the bacteria?

SET UP THE MICROTITER PLATE

1. **Look at the microtiter plate** on your desk. Keep it covered (maintain sterility). Along the top are numbers 1 -12 and down the sides are letters A – H.

1 2 3 4 5 6 7 8 9 10 11 12



www.apsnet.org/.../ bls/image/shadetree17.jpg

Figure 1. A microtiter plate

2. **Label the plate** on the edge. Use a code so you know what antibiotic is in each row. Record the row designation for each antibiotic in your notebook.
3. **Dispense 125 µl** of your nutrient broth-bacteria mixture (tube D) into rows A- D, wells 1-12.
4. **Dispense 125 µl** of <u>sterile</u> nutrient broth into well H1. This will be used to “blank” the spectrophotometer.
5. **Dispense 125 µl** of the tylosin into well A,1 using a sterile pipette tip (which pipette are you going to use?. There are now 250 µl in that well. Mix well by bringing the sample up and down in the tip.
6. **Remove 125 µl** from well A,1 and add it to well A,2. Mix well.
7. **Remove 125 µl** from well A,2 and add it to well A,3. Mix well
8. **Continue with the serial dilutions** through well A 11. DO NOT ADD ANY ADDITIONAL LIQUID TO WELL A 12. A 12 is a growth control. No antibiotic is added to this well.
9. **Repeat this process** with the other antibiotics.

Stock antibiotics (Original concentrations)

Tylosin	100 ug/ml	Erythromycin	10 ug/ml
Azithromycin	10 ug/ml	Roxithromycin	100ug/ml

THE NUMBERS IN THE “WELLS” BELOW (SHOWN AS BOXES) REPRESENT THE **CONCENTRATION OF THE ANTIBIOTIC** IN THAT WELL.

CONCENTRATION IS ug/mL (micrograms/milliliter)

Well #	1	2	3	4	5	6	7	8	9	10	11	12
T	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.099	0.049	Control
E	5	2.5	1.25	0.6255	0.312	0.156	0.078	0.039	0.019	0.009	0.0049	Control
A	5	2.5	1.25	0.6255	0.312	0.156	0.078	0.039	0.019	0.009	0.0049	Control
R	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.099	0.049	Control
	Blank											

Figure 2. A representative of MIC data (T = tylosin, E = erythromycin, A = azithromycin, R = roxithromycin, Blank= broth only)

COLLECT THE DATA.

1. **The next day,** one group member needs to return to read the plate. Please sign up for a time before you leave lab.
2. **Interpret the data**. Look at the print out of absorbencies of the wells. The machine is set up to "ignore" the background color of the medium. It will only record how much light can be absorbed by the bacteria growing in the nutrient broth.
3. **Figure out the MIC**. The MIC is the first well where growth of bacteria is inhibited.

Well	1	2	3	4	5	6	7	8	9	10	11	12
T	0.001	0.002	0.002	0.046	0.089	0.224	0.345	0.347	0.359	0.382	0.399	0.389
E	0.002	0.009	0.066	0.068	0.056	0.049	0.055	0.068	0.345	0.347	0.359	0.378
A	0.002	0.009	0.066	0.068	0.056	0.049	0.055	0.068	0.375	0.344	0.389	0.394
R	0.002	0.009	0.066	0.068	0.056	0.049	0.055	0.068	0.298	0.304	0.311	0.384

*Well H1 should contain only broth----

Figure 3. Optical density of bacteria in wells, as read on a microtiter plate reader.
(T = tylosin, E = erythromycin, A = azithromycin, R = roxithromycin)

Figure out the MIC. The MIC is the first well where growth of bacteria is inhibited.

Growth of bacteria is represented as absorbance in each well. The higher the number, the more bacteria there are. If there are bacteria growing, the absorbance reading will be over 0.1.

Look at the first row with Tylosin. Notice the absorbance in well 5 is 0.089 and well 6 is 0.224 (Figure 2, above). We can use the differences in absorption to distinguish between growth and no growth. In well 5, there is NO growth of bacteria, meaning that concentration of Tylosin (6.25 ug/mL) killed the bacteria. Then look at well 6. It has bacteria growing in it. That concentration of Tylosin did NOT kill the bacteria.

The **MIC** is determined by looking at these two wells (5 and 6). Well 5 contains 6.25 ug/mL of Tylosin and it is the Minimal Inhibitory Concentration.

How precise do you think this value is? Can you think of an additional experiment that would provide more precision?

RECORD YOUR DATA: Construct a table where you can put the antibiotics tested and the MIC value.

START THE INDUCTION STUDY (week of 12 Mar): Can bacteria be "induced" to greater resistance by growing in the presence of the antibiotic?

1. **Dispense** 2.0 ml nutrient broth to 15 tubes. Be careful! YOU do not want to contaminate the broth with other bacteria.
2. **Prepare the antibiotic.** In order to investigate whether or not this bacterium can be induced, we need to grow the bacteria in the presence of the antibiotic. The MIC for tylosin was:________________________. What was the highest tylosin concentration that allowed the bacteria to grow?________________________________. We want to add less than that concentration of tylosin to each of our tubes.
   Assume that the MIC for your microbe was 0.97 µg/ml. That means that the highest concentration of tylosin where the microbe could grow was 0.488 µg/ml. Since you have 2 ml in each tube you need 0.400 µg/ml X 2 ml = 0.800 µg of tylosin or less.
3. **Look at the concentration of tylosin**. Calculate how many µl's you will need to dispense the tylosin concentration you need for sub-MIC induction.
4. **Label your 15 tubes**. Each tube's label should contain your initials, Day1,or 2 or 3 up to day 14, the amount of tylosin added and your lab section. One tube should be labeled as "growth control."
5. **Add that amount of tylosin** to 14 tubes. DO not any tylosin to your growth control tube. Why?
6. **Inoculate your Day 1 tube and growth control tube** with 10 µl of an overnight culture provided for you. DO NOT add the bacteria to any other tube!
7. **Place the tubes in the 37^{o}C incubator.** You should place 2 tubes in the incubator. One tube is the growth control tube and the other tube is Day 1 induced.
8. **Come back daily** for 13 days. In your group divide the responsibility so only one person comes back each day. You will transfer 10 µl of the grown culture from Day 1 to tube labeled Day 2. If you do not have growth in either of your tubes notify the instructor immediately. The Day 1 tube should be placed in a discard rack in the lab. The next day you will transfer 10 µl of the grown culture from Day 2 to Day 3. Keep going with the transfers for 13 days.

WERE THE BACTERIA INDUCED? How do we know? We repeat the MIC's. Refer to "Determining the base-line MIC…." Page 2 and follow that protocol.

DATA COLLECTION AND ANALYSIS:

Record the MIC's for post-induction. Compare the data.

Antibiotic	Baseline MIC µg/ml	MIC (µg/ml) post induction
Tylosin		
Erythromycin		

Conclusions.

Guidelines for this report:

--Individual work: We expect you to do this entire assignment on your own. While we encourage you to discuss the data (and significance) with your friends, the final submitted product (construction of tables, calculations, text) MUST be your own.
--Typed---no electronic copies will be accepted
--Cover page----Name, title of lab, pledge
--30points
--due week of April 9. The third test is April 6. Plan accordingly

Introduction

- Antibiotic resistance---definition, relevance
- Macrolides---class, uses, examples
- How are microbes resistant to macrolides: Take the role of the bacteria. What must the bacteria due to resist these antibiotics (at the MOLECULAR level)?
- Induction---process and application
- Rationale behind experiment
- Purpose of experiment

Results

- Pre-MIC results: Table and text
- Post-MIC results: Table and text
- if your pre- and post- MIC data were not usable, there will be data on Blackboard
    - include a DISCLAIMER---if other data is being used
    - no need to include copy of this data---just the MIC's

Discussion

- What was the purpose of the experiment?
- Did the induction work? What data supports this?
- Speculate on what has happened in the bacteria (at the molecular level)
- If you had un-usable data, compare it to the data on Blackboard
    - What do you think you did wrong?
- What improvements would you make?
- What would be **THE** next **EXPERIMENT**?

References: Use 3 – 4 print references. You can use web sites if they are in addition to print sources.

- For introduction and/or discussion---tie in reference
- Include bibliography

References:

http://www.greatvistachemicals.com/industrial_and_specialty_chemicals/tylosin.html
www.apsnet.org/.../ bls/image/shadetree17.jpg
Brady M S [Reprint author]; Strobel R J [Author]**;** Katz S E [Author]. In-Vitro Analytical System the Abillity of Antibiotics at Residue Levels to Select for Resistance in Bacteria [Article] *Journal of the Association of Official Analytical Chemists. 71(2). 1988. 295-298*
Salyers, A.A, and D.D. Whitt. Revenge of the Microbes. ASM Press 2005.

Kirby-Bauer Disk Diffusion Method

Goals:

- To understand one method to determine antibiotic resistance and sensitivity
- To interpret zones of inhibition

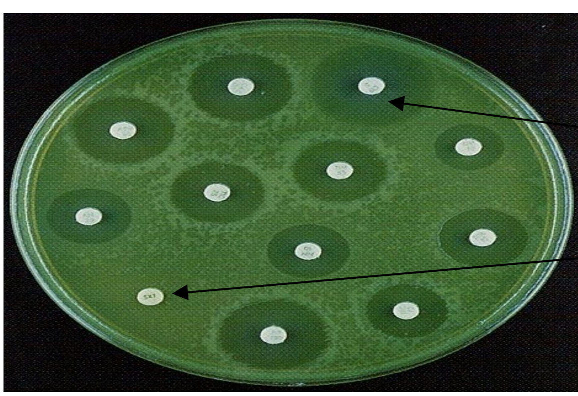
Introduction

Many people develop bacterial infections that need antibiotic treatment. In most cases, a physician treats "blindly" prescribing an antibiotic based on his best judgment. Under some circumstances, he will send a sample to a clinical laboratory for "culture and sensitivity." In the lab, the infectious agent is isolated and it is tested to a panel of antibiotics. Though these tests are automated today (and absorbance values are used to determine sensitivity and resistance just as we did in MIC's) years ago there was an alterative method, the disk diffusion method, used to gain the same outcome.

The Kirby-Bauer disk diffusion method relies on using filter paper disks impregnated with a set concentration of an antibiotic. There are many antibiotics samples available in this form so laboratories are able to test many different antibiotics simultaneously.

To run this assay, an overnight bacterial culture is used. A sterile swab is used to uniformly inoculate an agar plate. Once the sample has the opportunity to dry (about 5 min) individual antibiotic disks are placed on the agar surface. The agar plate is incubated (37^{o} C) overnight.

We interpret the agar plates by observing and measuring zones of inhibition. The filter paper disks are impregnated with a set concentration of one antibiotic. As it is placed on the agar, the antibiotic diffuses away from the disk. As the antibiotic diffuses away from the disk, the concentration of the antibiotic decreases. At the same time, the bacteria are beginning to grow. If the bacteria are sensitive to the antibiotic, their growth will be impeded. The magnitude of the growth inhibition will depend on the concentration of the antibiotic and the sensitivity of the bacteria being tested. The more sensitive the bacteria are to the antibiotic, the greater the inhibition even in the presence of small amounts of antibiotic (Fig. 1).



Pseudomonas aeruginosa

Sensitive – note the large zone of inhibition

Resistant – note the absence of a zone of inhibition

matcmadison.edu/.../ Unit%202/QCPseudCropped.jpg

Fig.1. Kirby-Bauer antibiotic susceptibility

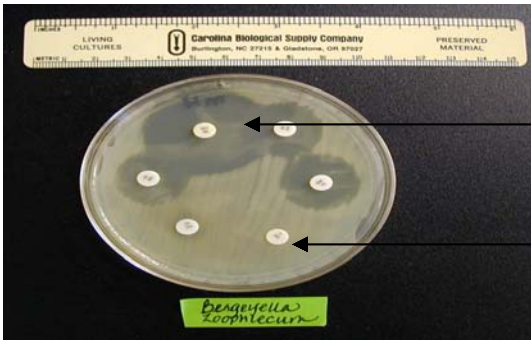
Lack of a zone of inhibition indicates that the bacteria tested can survive (and thrive!) in the presence of the highest concentration of antibiotic tested. The truly resistant bacteria are easily identified by no or little zones of inhibition. Conversely, those bacteria that are sensitive are readily seen by large areas of no growth. However, the results are not always that clear cut. There are some bacterial strains that exhibit growth that is not consistent with resistance yet are not truly sensitive. Their result is deemed "intermediate."

What we will do: Perform Kirby-Bauer antibiotic susceptibility studies

<u>Materials</u>

Overnight cultures of selected bacterial strains (concentration is approximately 2×10^9 cells per ml
Nutrient agar plates
Sterile saline
Sterile microfuge tubes
Sterile swabs
Antibiotic disks
Forceps
Alcohol burner
Rulers

1. **Label** 3 microfuge tubes A, and B.
2. **Dispense** 900µl of sterile saline into tubes A, and B.
3. **Dispense** 100µl from the original bacterial culture and place it into tube B. MIX well.
4. **Transfer** 100µl from tube A into tube B. MIX well
5. **Using tube B**, put the sterile swab into this tube, saturate it, and try to remove the excess fluid from the swab. What is the concentration of bacteria in tube B?
6. **Inoculate the agar plate** by swabbing over the surface of the agar plate in at least three directions. You want a uniform distribution of the bacteria on the agar plate.
7. **Let the plate** sit for 5 minutes to allow the bacterial suspension to dry on the plate.
8. **Place the disks containing** the antibiotics on the plate. Ensure that the forceps are sterile by dipping the forceps briefly in ethanol and passing them through a flame. WHEN USING AN ALCOHOL BURNER BE SURE THAT YOUR LAB BENCHES ARE CLEAR OF PAPERS, YOUR SLEEVES ARE ROLLED UP, AND HAIR IS TUCKED BACK.
9. **Incubate** the plate at 37^{o} C overnight.
10. **Collect the data.** Make a table in your notebook. Using a ruler measure (in mm) each zone surrounding the antibiotic disks (Fig. 2). Record that data in your table.



Note the large zone of inhibition indicating that the microbe is sensitive to that antibiotic.

Note the absence of a zone of inhibition indicating that the microbe is resistant to that antibiotic.

iws.ccccd.edu/.../ KB_B_zoo_ruler.JPG

Fig.2. Measuring zones on an antibiotic sensitivity plate

11. **Interpret your data.** Using the table provided, interpret the zones as being sensitive, intermediate, or resistant.

Table 1: Zone Diameter Interpretive Chart*

	Zone Diameter	**Interpretive Standards**	**(mm)**
Antibiotic	**Resistant**	**Intermediate**	**Susceptible**
Ampicillin	≤13	14-16	≥17
Cephalothin	≤14	14-17	≥18
Ciprofloxacin	≤15	16-20	≥21
Erythromycin	≤13	14-22	23
Kanamycin	≤13	14-17	≥18
Penicillin	≤14		≥15
Tetracycline	≤14	15-18	≥19

*provided with BD BBL™ Sensi-Disc™Antimicrobial Susceptibility Test Discs

Questions for thought:

1. Do you think the bacterial concentration used in swabbing the plates matters?
2. Why do the concentrations of the antibiotics used vary?
3. Which antibiotics tested would be acceptable to kill the bacteria?
4. Which antibiotics should not be used?
5. What does intermediate indicate? Should that drug be used for treatment?

Gel electrophoresis: How does it work?

We are going to use DNA gel electrophoresis in a simulation designed to differentiate genes found in monkey pox from similar genes in smallpox. Before we begin to utilize this technique it is important to become familiar with the materials we will be using. For gel electrophoresis we need to understand the relationship between the power supply and the gel box. This exercise will allow us to manipulate conditions and observe the results.

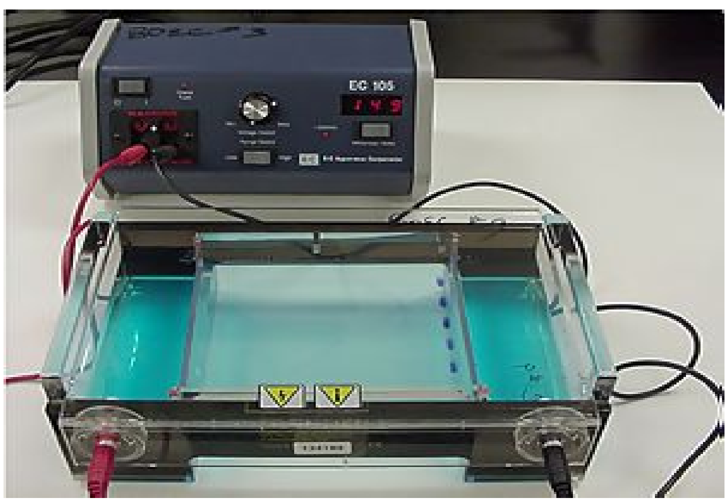
The power supply will provide the electrons that will flow into the gel box. The electrode where the electrons enter the gel box (black wire) is the **cathode** and is negative. The electrons leave the box and re-enter the power supply (through red wire) called the **anode**. Because electrons are moving from one pole to the other a potential energy difference is established between the two electrodes. This difference is called potential, and is measured in volts. This potential is responsible for the establishment of an electric field by which the ions in the gel box migrate. As these ions migrate, they produce an electrical current which is measured in amps (milliamps).

Based on your knowledge of DNA, would you load your sample at the anode of the cathode?
____

Why?

On your table is an electrophoresis set up. We will use gel electrophoresis to separate DNA molecules. Will charge result it the separation of different fragments of DNA?

How will different fragment of DNA be separated?



www.biotech.iastate.edu/.../ images/image08.jpg

Fig.1 Gel electrophoresis equipment

The three main components of a gel electrophoresis system are:

1. the power supply which provides electrons
2. the plastic gel box or the tank
3. a fluid of water and ions placed in the box

During electrophoresis, the current splits water into hydrogen ions (H^+) and hydroxyl (OH^-) ions. This process is electrolysis. Can you think of anyway to prove this is happening?

To begin: Examine the gel box and the power supply. It is designed that when the box is opened, the electrodes are disconnected.

CAUTION: Live current runs through the leads connected to the gel box. Be very careful doing the following simulations.

- When you want to remove the lid of the gel box, carefully use the small finger hold mounts. Do NOT pull on the wire leads to remove the lid.
- Since any wet surface can become conductive, it is advisable not to touch any part of the apparatus (gel box, wires) while the power supply is on. This is especially important if the outside of the box is wet, or if your hands are wet.

What you will do: Observe what happens in the gel box (& power supply read out) as we vary the conditions

Record all your data in your lab notebook.

1. Examine the power supply and identify the following:

on/off switch	switch between volts & milliamps
plugs for leads	digital display
voltage select dial	

2. Be sure the power supply is off. Then connect the empty box to the power supply. The red lead goes to the red port in the power supply and the black lead goes into the black port.

3. Set the lo/high switch to lo (if such a switch is present), turn the power supply on and select a potential of about 100V (volts). Record the current generated (measured in milliamps) in the empty box. Does current flow through air?

4. Turn the power supply off.

5. Open the gel box with power supply off (remember safety concerns) and add 300 ml of distilled water. Turn the power supply on. With a potential of 100V, record the level of current in the box. Does current flow through distilled water?

6. Turn the power supply off.

7. Add 1 ml of 1M NaCl to the distilled water in the box. Carefully mix the NaCl solution with the distilled water. Turn the power supply on and set the voltage to 100 V. Record the current. Does the current flow through distilled water that contains ions (Na^+, Cl^-)?

8. Choose 3 additional voltages and record the current at eachg voltage. Turn the power supply off.

When the current is flowing changes are occurring at the anode and the cathode. To review: at which electrode do electrons enter the gel box.______________.

At the cathode: 4 electrons + 4 H_20 $\longrightarrow$ 2 H_2 (gas) + 4 OH^-

At the anode: 4 H_20 $\longrightarrow$ O_2 (gas) + 4 H^+ + 2 H_20 + 4 electrons

Is there a pH difference at each electrode?

9. To demonstrate that a change in pH does occur during electrophoresis, we will use an indicator dye called phenol red. Empty the el box and add 300 ml of distilled water, 1 ml of 1 M NaCl, and 1 ml of phenol red. Carefully slosh the liquid in the gel box to get it equally mixed. What color is the liquid in the gel box?___________________________________

10. Turn the power supply on to 100 V and record the current. Record the time it takes for a color change to take place. Turn the power supply off.

    a. The accumulation of OH^- makes the solution basic. This can be confirmed by the phenol red turning pink. At what electrode do you see pink? _________ Based on the chemical reaction that is taking place at this electrode is this what you expected to see?________________________

    b. The accumulation of H^+ makes the solution acidic. This can be confirmed by the phenol red turning yellow (this is much paler than the pink color you see). At what electrode do you see yellow?__________ Based on the chemical reaction that is taking place at this electrode is this what you expected to see?________________________

11. Add 10 ml of 1X TBE (Tris ,Boric acid, EDTA) to the liquid in the gel box. Mix well. Does the color change any place in the solution? __________________What color is the liquid in the gel box?

12. Turn the power supply on again to 100V. Record the current. Record the time needed for color changes, if any. What might a lack of color change tell you about the effect of buffers on pH? _____________________________Turn the power supply off.

13. CLEAN UP:
    a. Unplug the power supply and disconnect the leads from the power supply.
    b. Empty the gel box and rinse thoroughly.
    c. Make sure your bench top is clean and orderly.

* Adapted from: http://www.accessexcellence.org/AE/AEPC/geneconn/gelbox/

Using DNA to Screen for Smallpox

Goals:

- To investigate how molecular biology techniques can be used to diagnose smallpox
- To use agarose gel electrophoresis to separate pieces of DNA

Introduction

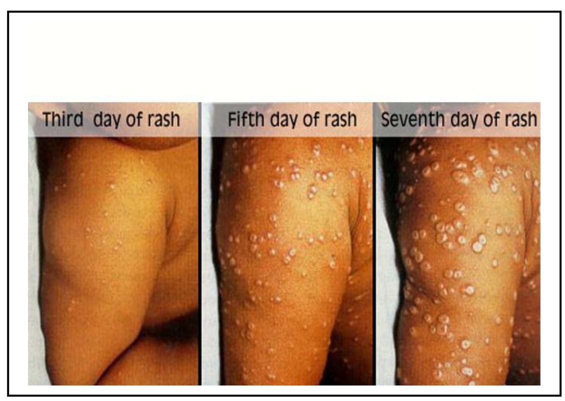
In this simulation, the FBI issues a terrorist alert to a **made-up city of Northwest** with a population of 1.5 million. Security is heightened at large gatherings and health care workers are advised as to the potential of a biowar break out. It is helpful to remember that many of the potential bioterror agents affects on human health have not been documented. Even those diseases that are not “novel” are so sporadic that most physicians are not familiar with the disease presentation and may make the appropriate diagnosis after the disease has progressed passed the time where effective treatment can be managed.

Shortly after the alert, a 27-year old Caucasian man enters an emergency room with a fever of 106^{o} F, and an exaggerated rash on his face and extremities. There is at least one family member with the same affliction. A short time earlier the man had attended a football game with over 80,000 people present.

The doctors are not sure what the problem is. However, in light of the terrorist alert, they placed the man in isolation. Their initial diagnosis (& fear) is smallpox. Preliminary tests are negative. Another physician who worked in Africa years earlier seems to think that the patient’s symptoms are very similar to monkeypox. The infectious disease team concurred. Further bolstering this diagnosis was the fact that the man’s brother-in-law worked at a local zoo that had recently received animals from the rainforest in the Democratic Republic of the Congo.

www.accesskent.com/.../ images/lyme_rash2.jpg

Fig. 1. Monkeypox



users.wfu.edu/turnaw2/ smallpox%20rash.jpeg

Fig. 2. Smallpox

So, the challenge is to diagnose the man and differentiate between smallpox (which could potentially be from a bioattack) and monkeypox, a zoonotic infection that is significantly less contagious. A sample is removed from a pustule and sent to the Center for Disease Control Laboratories for identification.

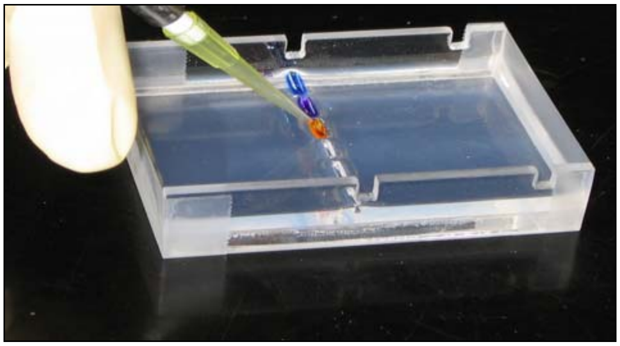
Molecular Biology to Differentiate Between Smallpox and Monkeypox (what level biosaftey do you think this sample is going to be handled in?)

In order to determine whether the infection is caused by smallpox or monkeypox, the DNA from the pustule is isolated. The goal of the experiment is to locate a particular gene that is found in these viruses. If the same exact gene was present in both diseases, that gene would not be able to differentiate between the two diseases. The gene that will be used is similar in both illnesses; however, it is a smaller piece of DNA in smallpox compared to monkeypox. Therefore, isolation of the genomic DNA, and that particular gene will result in classifying the infection as being smallpox or monkeypox.

Molecular biology techniques are quite sensitive but they require enough DNA for experimentation. The first task for the scientist is to extract the DNA. There are various methods that easily accomplish this task. Once the DNA is extracted, the experiment focuses on the presence (or absence) and size of a PARTICULAR piece of DNA. Looking at the entire genome is a daunting task and is untenable. Around 1990, a procedure was developed that allowed investigators to be able to "search" for the desired piece of DNA. This procedure is called the Polymerase Chain Reaction (PCR). In PCR, the particular piece of DNA is located (within the all the DNA present) by using primers. Primers are short pieces of DNA (from 20 – 40 nucleotides) that recognize segments of DNA on either side of the piece of DNA of interest. Think of primers as landmarks. Their role is simply to identify where the desired gene is. Other materials needed include dNTP's (the building blocks), reaction buffer and an enzyme (DNA polymerase) responsible for replicating the DNA.

After amplification of the DNA (in PCR), the piece of DNA can be visualized by agarose gel electrophoresis. In this technique the DNA samples are "loaded" into a well in an agarose gel submerged under running buffer. The current is turned on and the DNA migrates towards the positive end (why? Recall the structure of DNA). In this manner, pieces of DNA can be separated by size. Though size is not a conclusive identification of a gene, it provides preliminary data which can lead to further experiments and conclusive identification

What we will do: Agarose gel electrophoresis to differentiate between monkeypox and smallpox



biotrek.rdrake.org/ img/gallery/33electrophore...

Fig. 3. Loading an agarose gel

Adding the sample to the agarose gel:

The agaorse gel is 0.8% agarose.
Loading dye contains –
- Bromthymol blue
- Glycerol
- EDTA
- Tris buffer

1. **<u>Practice</u> loading a gel**. Next to each "practice" gel is a sample that contains loading dye and water. Before you load the sample, try loading between 35 – 38 µl of the sample into a well in the gel.
2. **Heat all the DNA samples** at 65^{o} C for two minutes. Allow the samples to cool for a few minutes before loading.
3. **Load the samples.** Load between 35 – 38 µl of each sample to lanes 1 -6. Tube A is dispensed into Lane1, tube B in lane 2…..and tube F in lane 7.

Tube	Sample
A	
B	
C	
D	
E	
F	

Table 1. DNA samples for gel electrophoresis

4. **Run the gel.** The gel will be electrophoreses at 125 V for 45 minutes.
5. **Stain the gel.** The DNA bands will be visualized due to the inclusion of ethidium bromide (EtBr) in the agarose gel. EtBr intercholates into the backbone of the DNA and appears pink under UV light. Be very careful around EtBr. It is classified as mildly carcinogenic (does this make sense to you?)
6. **Photograph the gel.** Pictures of the gel will be posted on Blackboard.

Data analysis:

1. Is this infection smallpox? How do you know?
2. What is meant by a DNA standard? Is a standard necessary for every gel you run?
3. Could you load the DNA samples if you forgot to add loading dye?
4. How can DNA be separated in an electric field?

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Disease Transmission Simulation - AIDS
Risk Assessment

Goals: As a result of this lab you will understand

1. To understand how disease is spread in a population.
2. To understand the role of simulation models in biology.
3. To discuss the social dimensions of AIDS.
4. Cannot assume 100% transmission of a disease.

Introduction:

Today's lab is designed to demonstrate the spread of a disease in a population. The disease could be any communicable disease; that is, a disease in which the disease-causing agent is passed from one individual to another either directly or indirectly. In our case we will assume the disease is AIDS.

In order to assess the rate of spread of the disease through a population and to determine the risk of becoming infected by AIDS, we will use a simulation model. Obviously, we cannot “pass” any type of pathogen in a laboratory setting. The simulation model will act as a “stand-in” for the real thing.

Procedure:

Each of you will be assigned a **stock solution** containing a clear liquid. All of the stock solutions are identical except one which is "infected" (not really infected, but rather a different clear liquid). Since no one knows who the infected individual is, we will make the following **assumptions**:

1. The infected individual has no symptoms - this is true of many of those infected with HIV (the virus that causes AIDS).

2. AIDS will not be transmitted in safe sex intercourse (i.e. AIDS can only be transmitted from a person with AIDS during unprotected sex).

The solutions in the bottles represent "bodily fluids" whose exchange is necessary for AIDS transmission. **CAUTION** - do not allow solutions to come in contact with your skin or clothing. These solutions contain weak acids and bases. They are not dangerous, but it is nevertheless good lab procedure to be careful. If you spill a solution on your skin, flood the area with running water from the tap.

SIMULATION #1: All individuals have an equal chance of getting infected (no protected sex at all) and only heterosexual contacts are allowed.

IMPORTANT: YOU WILL EXCHANGE "BODY FLUIDS" ONLY THREE TIMES! YOU WILL EXCHANGE WITH A DIFFERENT PERSON EACH TIME! WAIT FOR THE INSTRUCTORS SIGNAL TO EXCHANGE EACH TIME.

1. Obtain a test tube containing **stock solution** (at the front of the class) to a test tube. Label the test tube with your name. Make sure you write down the test tube number.

2. Round 1 contact: Find one person of the opposite sex at random. Each of you should remove 1 ml from your respective test tubes and place that 1 ml into the opposite person's test tube. Record the name of your first contact in the data Table on the next page. You will have 3 minutes to complete this exchange.

3. Round 2 contact: At the end of this time period, find a different partner (opposite sex) and again exchange 1 ml of solution. Record the name of the second contact.

4. Round 3 contact: After 3 minutes, repeat with a third contact (opposite sex). Record the name of the third contact.

5. After the three contacts are completed, use the phenol red solution provided to see if you are infected. To do this test, simply add phenol red to your test tube and record the color of your solution. Infected solutions are red; uninfected solutions are yellow.

6. Record the names of your contacts and the names of those who have become infected in the master Data Table. Now working with this information, determine who is the original carrier of AIDS in this lab population.

What does this tell you about transmission of a disease like AIDS among heterosexuals practicing unsafe sex?

DATA TABLE - SIMULATION #1
Record Name of Contact in Each Round

Name	Partner #1	Partner #2	Partner #3

SIMULATION #2: Drug Users Simulations

The assumptions here are that there can be both heterosexual and homosexual exchange via the sharing of needles. A second assumption is that there are two communities of people - those that inject drugs and those that do not inject drugs. Those that don't inject drugs also practice safe sex.

1. Obtain a second test tube containing a stock solution. Choose a different flask number and write it down.

2. Choose a different test tube than used in simulation 1. Odd-numbered test tube holders will exchange with odd-numbers and even with even.

3. Round 1 contact: Find one person that has an odd numbered test tube if your test tube has an odd number and even with even and exchange 1 ml of your respective **stock solutions** (as you did in Round 1, Simulation #1). Record the name of the person in the Data Table.

4. Round 2 Simulation: Exchange 1 ml with another person (odd, odd; even, even). Record name.

5. Round 3 Simulation: Exchange 1 ml with another person (odd, odd; even, even). Record name.

6. Record the names of your contacts and the names of those who have become infected in the master Data Table. Now working with this information, determine who is the original carrier of AIDS in this lab population.

What does this data tell you about transmission of a disease like AIDS among people in a population, some of whom inject drugs and some of whom do not?

DATA TABLE - SIMULATION #2

Record Name of Contact in Each Round			
Name	Partner #1	Partner #2	Partner #3

Survey Biology 110 Fall 2006

1. Bacterial pili are important for
a. locomotion
b. adhesion to target cells
c. DNA replication
d. phototaxis
e. flagellar growth

2. A missense mutation is the result of the
a. deletion of a large segment of DNA
b. insertion of 3 nucleotides
c. change in one nucleotide resulting in the change of a pivotal amino acid
d. change in one nucleotide resulting in the insertion of a stop codon
e. no change in the DNA but a change in the protein synthesis

3. Which of the following is NOT a site for antibiotics?
a. peptidiglycan
b. cell membrane
c. endoplasmic reticulum
d. DNA gyrase
e. tRNA synthase

4. Gram negative bacteria are different from Gram positive bacteria because Gram negative bacteria
a. have a thick peptidoglycan
b. lack an outer membrane
c. have two phospholipid layers
d. lack an endoplasmic reticulum
e. have extensive endoplasmic reticulum for the exportation of proteins

5. An antibiotic that was designed to interfere with translation would target
a. the ribosomes
b. DNA gyrase
c. RNA polymerase
d. topoisomerase
e. none of the above

6. A non-specific mechanism employed by bacteria to be resistant to an antibiotic is
a. production of an enzyme to destroy the antibiotic
b. to change the shape of the target of the antibiotic
c. an efflux pump
d. alteration of the ribosome binding site for the antibiotic
e. none of the above

7. Bacterial exchange of DNA by cell-to-cell contact is
a. transformation
b. conjugation
c. transduction
d. transcription
e. fission

8. The pathology of malaria is due to:
a. exflagellation
b. the *Anopheles* mosquito
c. parasite multiplication in red blood cells
d. the parasite causing red blood cells to sickle
e. fever and chills

9. The superior fitness of people who are heterozygotes for the hemoglobin gene is called:
a. heterosis
b. a balanced polymorphism
c. a mutation
d. hemolysis
e. imbalanced inheritance

10. There are three lines of defense in the immune system—the first "layer" is composed of physiological and physical barriers. Which of the following is NOT part of the barrier?
a. white blood cells
b. epithelial layer of skin
c. tears
d. mucus
e. sebum

11. Which of the following is NOT a characteristic of the inflammatory reaction?
a. redness
b. swelling
c. tenderness
d. pus
e. warmth

12. What type of lymphocyte is associated with antibody-mediated acquired immunity?
a. B lymphocytes
b. G lymphocytes
c. M lymphocytes
d. P lymphocytes
e. T lymphocytes

13. What type of cells will be produced AFTER a lymphocyte has encountered an antigen?
a. plasma cells
b. helper cells
c. cytotoxic cells
d. memory cells
e. killer cells

14. Cell associated lipopolysaccharide toxins are
a. associated only with Gram positive bacteria
b. endotoxins
c. exrtracellular toxins
d. only associated with *E. coli*
e. found in the cytoplasm of selected bacteria

15. The introduction of foreign genes into bacteria by a bacteriophage is called
a. transformation
b. conjugation
c. transductio
d. spontaneous exchange
e. phageogenesis

16. A nucleotide (in DNA) is composed of
a. phosphate, base and sugar
b. phosphate and base
c. thymine, d-ribose and a phosphate
d. phosphate and sugar
e. adenine, ribose and a phosphate

17. An example of a macrolide antibiotic is
a. erythromycin
b. tetracycline
c. pencillin
d. cephalothin
e. streptomycin

18. A retrovirus
a. is smallpox
b. has to convert DNA into RNA in order to be effective
c. has DNA and RNA in its' genome
d. has an RNA genome
e. has a capsid and an envelope

19. Viruses enter target cells by "docking onto"
a. flagella
b. specialized receptors on the host cell membrane
c. the ribosomes
d. the capsid
e. the cell envelope

20. The R_o value
a. is related to the size of the infecting agent
b. indicates the number of deaths in a population
c. decreases with transmission rate as well as the duration of the infection
d. depends directly on the density of susceptible hosts
e. is used by drug companies to predict the success of a new antimicrobial agent

21. Infectious diseases can be transmitted by
a. fomites
b. direct contact
c. transient contact
d. vectors
e. all of the above

22. One reason that the bacteria that causes Bubonic plague is so successful at infection is
a. because of its thick peptidoglycan
b. the resilience of its' spores and easy spread
c. significant resistance to many antibiotics
d. the great number of flagella
e. its cytotoxic effect on macrohages

23. Apoptosis is
a. symptomatic of Bubonic plague
b. programmed cell death
c. symptomatic of Sylvatic plague
d. due to the Gram positive composition of the infecting bacteria
e. only seen in pneumonic plague

24. The first epidemiologist ________________ studied a _____________________outbreak in London.
a. John Snow, cholera
b. Fred Griffith, pneumonia
c. John Snow, pneumonia
d. Fred Griffith, cholera
e. John Snow, tuberculosis

25. *Vibrio cholera* produces an _________________ which is responsible for the characteristic_____________ associated with the disease.
a. exotoxin, redness and bleeding
b. enterotoxin, attack on the mucosal epithelium causing severe diarrhea
c. apoptosis, killing of mucosal cells
d. exotoxin, attack on mucosal cells causing severe diarrhea
e. endotoxin, redness and bleeding

26. In order for HIV to be successful in establishing an infection it must be able to produce
a. glycocalyx
b. pili
c. reverse transcriptase
d. an envelope
e. spikes

27. The Tuskegee study involved examining
a. college populations in Alabama and their susceptibility to syphilis
b. the infectious cycle of syphilis in men and women across Alabalma
c. syphilis infection rates in African-American males
d. the course of syphilis in African-American men that were not treated with antibiotics
e. syphilis rates in prisons

28. The function of gp120 is to bind to
a. gp41
b. the CD4 receptor on human cells
c. the spikes on HIV
d. the outer envelope of HIV
e. DNA

29. The DNA version of the HIV genome gains access to host DNA by the action of
a. gp120
b. reverse transcriptase
c. APOBEC3G
d. integrase
e. nef

30. *Mycobacterium tuberculosis* is a(n)
a. Gram positive microbe
b. facultative intracellular parasite associated with macrophages
c. microbe with a very thick lipopoysaccharide layer that assists in its virulence
d. motile rod able to penetrate the lungs quickly
e. microbe that grows very fast making it very virulent

31. Rifampin is an effective anti TB drug as it interrupts
a. replication
b. cell division
c. DNA synthesis
d. translation
e. transcription

32. *Mycobacterium tuberculosis* differs from other microbes in that it does not
a. have a cell membrane
b. require any special nutritional requirements
c. stain by the Gram reaction and is called acid fast
d. replicate its own DNA
e. grow in culture

33. *Mycobacterium tuberculosis* is difficult to treat because the microbe
a. needs long term antibiotic treatment and patients are not always compliant
b. is a spore former and can resist antibiotics
c. has a thick peptidoglycan and the antibiotics cannot penetrate this layer
d. grows very quickly and overcomes the drugs
e. has a thick outer covering composed of lipid

34. Transmission of variola virus is enhanced because this virus
a. easily spreads through bed sheets
b. is small and able to disseminate easily
c. has a thick lipid covering to protect it from the environment
d. is highly stable and remains infective for long periods of time outside of the host
e. attaches to bacteria normally found in the upper respiratory tract and enters the body with the bacteria

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b. rodents
c. mosquitos
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e. breaks down the host cell membrane allowing the emergence of newly formed viral particles

42. Serial dilution is a process that
a. results in a lower and lower number of bacterial colonies on an agar plate
b. uses DNA to transform bacteria into another form
c. is not efficient and is no longer an acceptable laboratory technique
d. cannot be done in routine college laboratories
e. can only be done using large volumes of buffer and sample

43. The purpose of the minimal inhibitory concentration is to
a. investigate the number of bacteria in a sample that can kill a person
b. look at various disinfectants commonly used
c. determine the lowest concentration of an antibiotic that can kill a bacterial strain
d. find out the smallest number of bacteria that can cause an epidemic
e. look at how different microbes interact

44. If you want to dispense 200 µl of a fluid which pipetttor(s) would you use?
a. P20
b. P200
c. P1000
d. P20 or P200
e. P200 or P1000

45. In DNA gel electrophoresis DNA is separated due to
a. the salt concentration in the buffer
b. the density of the agarose that the DNA is being pulled through
c. the positive charge
d. its size as smaller fragments migrate faster
e. its large size

46. HIV phylogeny can show
a. how virulent a strain of HIV is
b. the relatedness between strains of HIV isolated from different individuals
c. which antiviral compounds will be effective
d. how quickly the person will develop AIDS

47. In lab, smallpox and monkey pox were differentiated by
a. looking at different proteins
b. examining pictures of patients and noting their rashes
c. using agarose gel electrophoresis
d. a computer simulation
e. examining phylogeny trees

48. ELISA tests look for
a. an antibody to a specific antigen
b. macrophages
c. an antigen circulating in the bloodstream
d. an increased number of white blood cells
e. all antibodies a person has made

49. Herd immunity implies that
a. a substantial percentage of the population needs to be vaccinated
b. cattle have been vaccinated against mad cow disease
c. a program will be initiated to begin vaccinations
d. the older generation has already been vaccinated
e. a substantial percentage of the population has been vaccinated and will protect those not immunized

50. Morbidity and mortality charts monitor the
a. development of an infection and quick recovery
b. incidence of a disease in a population and those that die
c. speed of infection and development of infection
d. spread of the disease and the number that develop complications
e. speed of infection and the spread of disease

1. Bacterial pili are important for
a. locomotion
b. adhesion to target cells
c. DNA replication
d. phototaxis
e. flagellar growth

2. A missense mutation is the result of the
a. deletion of a large segment of DNA
b. insertion of 3 nucleotides
c. change in one nucleotide resulting in the change of a pivotal amino acid
d. change in one nucleotide resulting in the insertion of a stop codon
e. no change in the DNA but a change in the protein synthesis

3. Which of the following is NOT a site for antibiotics?
a. peptidiglycan
b. cell membrane
c. endoplasmic reticulum
d. DNA gyrase
e. tRNA synthase

4. Gram negative bacteria are different from Gram positive bacteria because Gram negative bacteria
a. have a thick peptidoglycan
b. lack an outer membrane
c. have two phospholipid layers
d. lack an endoplasmic reticulum
e. have extensive endoplasmic reticulum for the exportation of proteins

5. An antibiotic that was designed to interfere with translation would target
a. the ribosomes
b. DNA gyrase
c. RNA polymerase
d. topoisomerase
e. none of the above

6. A non-specific mechanism employed by bacteria to be resistant to an antibiotic is
a. production of an enzyme to destroy the antibiotic
b. to change the shape of the target of the antibiotic
c. an efflux pump
d. alteration of the ribosome binding site for the antibiotic
e. none of the above

7. Bacterial exchange of DNA by cell-to-cell contact is
a. transformation
b. conjugation
c. transduction
d. transcription
e. fission

8. The pathology of malaria is due to:
a) exflagellation
b) the *Anopheles* mosquito
c) parasite multiplication in red blood cells
d) the parasite causing red blood cells to sickle e) fever and chills

9. The superior fitness of people who are heterozygotes for the hemoglobin gene is called:
a) heterosis
b) a balanced polymorphism
c) a mutation
d) hemolysis
e) imbalanced inheritance

10. There are three lines of defense in the immune system—the first "layer" is composed of physiological and physical barriers. Which of the following is NOT part of the barrier?
a) white blood cells
b) epithelial layer of skin
c) tears
d) mucus
e) sebum

11. Which of the following is NOT a characteristic of the inflammatory reaction?
a) redness
b) swelling
c) tenderness
d) pus
e) warmth

12. What type of lymphocyte is associated with antibody-mediated acquired immunity?
a) B lymphocytes
b) G lymphocytes
c) M lymphocytes
d) P lymphocytes
e) T lymphocytes

13. What type of cells will be produced AFTER a lymphocyte has encountered an antigen?
a) plasma cells
b) helper cells
c) cytotoxic cells
d) memory cells
e) killer cells

14. Cell associated lipopolysaccharide toxins are
a. associated only with Gram positive bacteria
b. endotoxins
c. exrtracellular toxins
d. only associated with *E. coli*
e. found in the cytoplasm of selected bacteria

15. The introduction of foreign genes into bacteria by a bacteriophage is called
a. transformation
b. conjugation

c. transduction
d. spontaneous exchange
e. phageogenesis

16. A nucleotide (in DNA) is composed of
a. phosphate, base and sugar
b. phosphate and base
c. thymine, d-ribose and a phosphate
d. phosphate and sugar
e. adenine, ribose and a phosphate

17. An example of a macrolide antibiotic is
a. erythromycin
b. tetracycline
c. pencillin
d. cephalothin
e. streptomycin

18. A retrovirus
a. is smallpox
b. has to convert DNA into RNA in order to be effective
c. has DNA and RNA in its' genome
d. has an RNA genome
e. has a capsid and an envelope

19. Viruses enter target cells by "docking onto"
a. flagella
b. specialized receptors on the host cell membrane
c. the ribosomes
d. the capsid
e. the cell envelope

20. The R_o value
a. is related to the size of the infecting agent
b. indicates the number of deaths in a population
c. decreases with transmission rate as well as the duration of the infection
d. depends directly on the density of susceptible hosts
e. is used by drug companies to predict the success of a new antimicrobial agent

21. Infectious diseases can be transmitted by
a. fomites
b. direct contact
c. transient contact
d. vectors
e. all of the above

22. One reason that the bacteria that causes Bubonic plague is so successful at infection is
a. because of its thick peptidoglycan
b. the resilience of its' spores and easy spread

c. significant resistance to many antibiotics
d. the great number of flagella
e. its cytotoxic effect on macrohages

23. Apoptosis is
a. symptomatic of Bubonic plague
b. programmed cell death
c. symptomatic of Sylvatic plague
d. due to the Gram positive composition of the infecting bacteria
e. only seen in pneumonic plague

24. The first epidemiologist ________________ studied a ______________________outbreak in London.
a. John Snow, cholera
b. Fred Griffith, pneumonia
c. John Snow, pneumonia
d. Fred Griffith, cholera
e. John Snow, tuberculosis

25. *Vibrio cholera* produces an _________________ which is responsible for the characteristic_____________ associated with the disease.
a. exotoxin, redness and bleeding
b. enterotoxin, attack on the mucosal epithelium causing severe diarrhea
c. apoptosis, killing of mucosal cells
d. exotoxin, attack on mucosal cells causing severe diarrhea
e. endotoxin, redness and bleeding

26. In order for HIV to be successful in establishing an infection it must be able to produce
a. glycocalyx
b. pili
c. reverse transcriptase
d. an envelope
e. spikes

27. APOBEC3G is
a. produced by HIV and helps in establishing an infection
b. lethal to HIV and will kill the virus
c. assists HIV in gaining entry into T4 cells
d. a protein found inside cells that has an antiviral role
e. needed by host cells to accept the HIV virion into the cell

28. The function of gp120 is to bind to
a. gp41
b. the CD4 receptor on human cells
c. the spikes on HIV
d. the outer envelope of HIV
e. DNA

29. The DNA version of the HIV genome gains access to host DNA by the action of
a. gp120

b. reverse transcriptase
c. APOBEC3G
d. integrase
e. nef

30. *Mycobacterium tuberculosis* is a(n)
a. Gram positive microbe
b. facultative intracellular parasite associated with macrophages
c. microbe with a very thick lipopoysaccharide layer that assists in its virulence
d. motile rod able to penetrate the lungs quickly
e. microbe that grows very fast making it very virulent

31. Rifampin is an effective anti TB drug as it interrupts
a. replication
b. cell division
c. DNA synthesis
d. translation
e. transcription

32. *Mycobacterium tuberculosis* differs from other microbes in that it does not
a. have a cell membrane
b. require any special nutritional requirements
c. stain by the Gram reaction and is called acid fast
d. replicate its own DNA
e. grow in culture

33. *Mycobacterium tuberculosis* is difficult to treat because the microbe
a. needs long term antibiotic treatment and patients are not always compliant
b. is a spore former and can resist antibiotics
c.   has a thick peptidoglycan and the antibiotics cannot penetrate this layer
d. grows very quickly and overcomes the drugs
e. has a thick outer covering composed of lipid

34. Transmission of variola virus is enhanced because this virus
a. easily spreads through bed sheets
b. is small and able to disseminate easily
c. has a thick lipid covering to protect it from the environment
d. is highly stable and remains infective for long periods of time outside of the host
e. attaches to bacteria normally found in the upper respiratory tract and enters the body with the bacteria

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42. Which of the following viruses are RNA viruses?
a. HIV , West Nile, Ebola and Influenza
b. HIV, Herpes, West Nile and Ebola
c. Influenza, Ebola, Smallpox and HIV
d. Hepatitis B, Ebola, West Nile and HIV
e. Hepatitis B, Herpes, Smallpox and Influenza

43. *E. coli* 0157:H7 is
a. found as a normal inhabitant of the intestinal tract
b. unable to survive in the environment making it no threat to human health

c. causes a mild infection in all patients including young children and the elderly
d. has acquired a Shiga-like toxin which can lead to hemorrhagic colitis in a small number of cases
e. is the cause of Mad Cow disease

44. Though *Staph aureus* is commonly found on the skin, infection with MRSA is
a. serious as this strain is resistant to many antibiotics including methicillin
b. not found in the US but is limited to developing countries
c. easily treated as the bacteria is sensitive to methicillin
d. limited to the upper respiratory tract as it is only transmitted in air droplets
e. limited to moist areas of the skin

45. The hyaluronic acid capsule of *Streptococcus pyogenes* assists in
a. phagocyttosis by white blood cells
b. adhesion to receptors on white blood cells
c. the release of toxins that destroy red blood cells
d. secondary infections such as rheumatic heart disease
e. hiding its foreign antigens from the host thereby going undetected

46. The "flesh eating" bacteria is the same bacteria that commonly causes
a. sexually transmitted diseases like syphilis
b. strep throat
c. hemolytic diarrhea
d. hemolytic urinary tract infection
e. boils and pimples

47. The reservoir for the West Nile Virus is
a. bats
b. birds
c. mammals
d. pigs
e. there is no know reservoir for this virus

48. Hanta viruses replicate exclusively in the cellular
a. cytoplasm
b. nucleus
c. endoplasmic reticulum
d. vacuole
e. cell membrane

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