Disclaimer

During the course of this laboratory exercise, students will simulate the release of pathogenic bacteria. Using various laboratory techniques, they will attempt to characterize, identify and then treat the pathogen. However, this is merely a SIMULATION. No harmful pathogens will be used during the course of this exercise. All additional material and chemicals will be used under the supervision of trained lab personal.

Activity summary

Students well play the role of a prospective scientist/lab technicians interviewing for a job at the "Boston University Center for Disease Control" (BUCDC). During the course of orientation program, "pathogenic" bacteria will be accidently "released" into the lab. Over the next 3 hours, students will attempt to model, identify and then "treat" the outbreak using basic laboratory skills include, microscopy, catalase tests and gram staining. Additional post and pre-lab material is enclosed in this packet to be used at the teachers' discretion.

Logistics

Location: Metcalf Science Center

Address: 590 Commonwealth Avenue, Boston MA 02215

<u>Time:</u> Please arrive at 9:00 AM sharp <u>**Transportation:**</u> The easiest access to Boston University is via the Green Line (B Line). The Blandford Street T stop is a short walk to the Metcalf Science Center. Although metered parking (4 hours at 15 min per quarter) may be found on the street, this option is discouraged <u>**Room Location**</u>: Biology lab classrooms have been reserved for this activity. Each lab can accommodate (24) students. Therefore, please RSVP with the number of perspective students



so that there is enough space and material for each student. **Who to invite**: This lab is designed for high school students who have taken or are

currently enrolled in either A.P. or regular biology class.

How To Prepare: Pre-lab material is included in this packet to be used at the teachers' discretion. However, it is strongly advised that teachers use the microscope tutorial (see references below) in order to familiarize students with this piece of scientific equipment. **What to bring:** No outside material is required for this activity with the exception of a pen or pencil. Also please note that lab space is limited and if possible students should be encouraged to leave their backpacks at home. Furthermore, students should be encouraged to wear close toed shoes with rubber souls.

Post Activity Reception: A pizza lunch will be provided to the students upon completion of the activity.

Experiences in Sciences Panel: The panel will be composed of, professors, graduate students, undergraduates and others who have a career in sciences. The composition of the panel may change depending on the day, but a number of disciplines will be represented including; chemistry, physics, computer science et.

Schedule of Activities

- 9:00am Welcome/Intro: 5 min
 - Boston University staff and students will briefly introduce themselves
- 9:05 am Safety Lessons: 10 min
 - A brief comment on lab safety. The policies touched upon can be applied to any lab
- 9:15am Modeling A Synthetic Epidemic: 45 min
 - Using the glow germ powder, students will create a synthetic epidemic and attempt to identify how the pathogen was passed and who was initially exposed to
- 10:00 am Gram Stain And Microscopy: 60 min
 - Identification of bacteria via shape and gram staining microscopes
- 11:00 am- Catalase test: 20 min
 - Further identification of bacteria via catalase activity
- 11:20 am Antibiotic sensitivity: 30 min
 - Students will identify the best treatment of the outbreak based upon resistance to antibiotics
- 11:50 am Close: 10 min
 - Students will be left with a number of questions which will serve to prompt classroom discussion and connect the Outbreak activity with the regular biology curriculum
- 12:00 pm Lunch and Panel Discussion

Section 1 Lab Safety/Synthetic Outbreak

Activity Outline

- Lab safety overview
- Disease transmission methods
 - Skin to skin, ingestion, inhalation, bodily fluids
- Modeling of a synthetic epidemic
 - Disguised as an icebreaker, students will shake hands passing a fluorescent chemical around the room. Students will then identify who has been "infected" and attempt to establish how the pathogen was passed and who was the first person infected (patient zero).
- Identification of Patient Zero

Introduction for the Teacher: Lab Safety

Lab safety centers around 2 primary goals

- 1. Protect the individual from any dangerous material
- 2. Protect the sample from the individual

This is accomplished via the use of appropriate lab attire (gloves, goggles and lab coats).

Review of Lab Safety Protocols

- No food, drink gum chewing or cell phone usage in the lab
- Appropriate clothing shall be used at all times
 - Goggles, lab coats, gloves, closed toed shoes
- Tie hair back
- Wash bench with 70% ethanol
- The instructor should be noted of any spills
- All trash should be disposed of in the appropriate bins

Introduction for the Teacher: Synthetic Epidemic

The first known case of a disease is defined as "patient zero," examples include Mary Mallon (AKA Typhoid Mary) and, allegedly, Gaëtan Dugas (AIDs). Interactions between these individuals and those around them lead to spreading of the disease through a population. The method through which a pathogen can infect others include, skin-toskin contact, inhalation, ingestion and the exchange of bodily fluids

The actual outbreak may then be classified a number of different ways. Endemic diseases tend to be a constant in a particular population, but are present at low levels. However, if this same disease approaches or exceeds those levels, it maybe classified as an epidemic. If unchecked and the epidemic becomes a global problem, it may be referred to as pandemic.

Diseases may also be classified as emerging or re-emerging depending on their history. Emerging diseases includes AIDS and SARs which are either are the result of newly identified microbes. Re-emerging diseases are the products of known microbes which have moved to new geographic locations/populations or have re-infected the same population as the result of a genetic or ecological change.

Pre-Lab Activity: Lab Safety

Using the cartoon below, discuss what is considered safe laboratory behavior. The question listed below may serve as a starting point

- 1. List 3 unsafe activities shows in the illustration and explain why each is unsafe.
- 2. List 3 correct lab procedures depicted in the illustration.
- 3. What should Bob do after the accident?
- 4. What should Sue have done to avoid an accident?
- 5. Compare Luke and Duke's lab techniques. Who is following the rules?
- 6. What are three things shown in the lab that should not be there?
- 7. Compare Joe and Carl's lab techniques. Who is doing it the correct way?
- 8. List three items in the illustration that are there for the safety of the students in the lab.
- 9. What is Betty doing wrong?^{*}

^{*}Taken from the "Biology Corner" <u>http://www.biologycorner.com/</u>



References & Links

Lab Safety for High Schools: A basic outline for lab safety for a high school biology class

(http://www.chem.unl.edu/safety/hslab7.html)

Boston University Office of Environmental Health and Safety: References for lab safety

(http://www.bu.edu/research/compliance/oehs/index.shtml)

Flinn Scientific: Detailed overview of various aspects of lab safety including chemical storage and eye wear.

(http://www.flinnsci.com/Sections/Safety/safety.asp)

YouTube Lab Safety Videos Video 1 (<u>http://www.youtube.com/watch?v=Kn5XfHHED9c</u>) Video 2 (<u>http://www.youtube.com/watch?v=56HEoOiXF7c&feature=related</u>)

Sample Lab Safety Contract (http://shs.westport.k12.ct.us/mjvl/lazaroff/lab_safety_agreement.doc)

Pre-Lab Activity: Synthetic Epidemic Discussion/Simulation Real Life Examples of Outbreaks

The Boston University Outbreak activity was inspired by real world cases of both epidemics and pandemics. These historical cases are listed below along with links to various articles and reports. This material may serve both as a way to familiarize students with the concept of disease transmission and as means to stimulate in class discussions.

<u>Articles</u>

Diagram of Patient Zero: A graphical interpretation of how a single patient (patient zero) may lead to an epidemic (http://robslink.com/SAS/democd4/infect.htm

The Genesis of a Pandemic Influenza Virus: Study outline the progression of various outbeaks of influenza (http://linkinghub.elsevier.com/retrieve/pii/S0092867405010949)

Simulation of Pandemic Influenza Outbreak In Chicago: A study into a theoretical outbreak of influenza in Chicago (http://www.medicalnewstoday.com/articles/100195.php)

The TB Scare: A commentary focusing on the dangers of 1 individual infected with tuberculosis to the rest of the general public. (http://www.time.com/time/health/article/0,8599,1627159,00.html)

Tuberculosis Hazard on Long Airplane Flights: An analysis of the risks of infection in an enclosed environment (http://www.semp.us/publications/biot_reader.php?BiotID=439)

Center For Disease Controls FAQ for Swine Flue (http://www.cdc.gov/h1n1flu/)

Progression of Swine Flu Epidemic (http://biosurveillance.typepad.com/biosurveillance/2009/04/swine-flu-in-mexico-timeline-of-events.html)

Search for Patient Zero for Swine Flu (http://blogs.wsj.com/health/2009/04/29/search-for-swine-flus-patient-zero-leads-to-mexicanboy/?mod=sphere_ts&mod=sphere_wd)

<u>Simulations</u>

Pandemic: A virtual game in which students evolve a pathogenic agent and try to infect and kill the population of the world (http://www.crazymonkeygames.com/Pandemic-2.html)

Virus Outbreak Demo: A simulation of the spread of a virus through a defined population. Variables such as number of interactions, and infections rates can be modified

(http://vlab.infotech.monash.edu.au/simulations/networks/virus-outbreak/demo/)

Post Lab Questions

- 1. What are the two primary goals of lab safety?
 - Protect the individual from the sample and the sample from the individual
- 2. A student is working with a rob shaped, gram + bacteria. (S)he puts a small amount of liquid containing that bacteria on a plate and places it in an incubator to grow. The following day, the plate yields both gram + and cells. Using what you know regarding lab safety, what has happened to the sample?
 - Some type of contamination has occurred,
- **3.** Even if a sample is not harmful to the scientists why should they always wears gloves?
 - To protect the sample from any contamination from the scientists
- 4. In the outbreak scenario, when the pathogen was detected, the lab was pressurized to BELOW atmospheric pressure. Why is this so? (HINT: which direction will the air flow?)
 - If there is an airborne pathogen, and the lab is some one breached, air will flow into the lab, preventing the contaminated air from rushing out of the lab

- **5.** How does the advent of modern travel (cars/airplanes/trains/boats) influence the outbreak of a disease? (HINT: review the case of the TB patient traveling to Europe)
 - Containing the disease may be difficult as modern transportation allows for people to travel a great distance over a short period of time.
 Furthermore, they travel in groups, each person may become infected during a flight, carrying the pathogen to other destinations
- 6. Name three methods that a disease can be transmitted.
 - Skin to skin, bodily fluid, ingestion, inhalation.
- 7. During the Spanish Flu pandemic of 1918, those with the disease were isolated from the rest of the population. What is this called?
 - Quarantine
- 8. How would geographical boundaries such as rivers and mountains protect against the spread of a disease?
 - If people cannot overcome these boundaries, they effective provide a natural barrier resulting in quarantine.
- 9. How would the time that an infection takes to kill a carrier influence the spread of that pathogenic agent. (HINT: compare and contrast what would happen if the patient dies within 2 hours of infection, versus one that may not die for 2 years)
 - Disease that result in symptoms and or death early on, allow more time for intervention as the disease is visible. Furthermore, if the patient disease he/she may no longer pass the disease on. In this case the disease will burn itself out quickly. If the disease does not present any symptoms for a long time, then those infected are unaware and will continue to infect new people.
- 10. Draw a graph outlining the number of people infected over time for the pathogen used in this activity.
 - \circ Round 1=1 person
 - \circ Round 2=2 people
 - \circ Round 3=4 people
 - \circ Round 4=8
 - The growth is exponential

<u>Section 2 Gram Staining/Bacteria Morphological</u> Characterization/Microscope Tutorial

Activity Outline

- Classification of bacteria to identify the "unknown" pathogen
 - Gram staining
 - Bacterial morphology
- Microscopy

Introduction for the Teacher: Gram Staining

Invented in 1884 by Hans Christian Gram as a means to differentiate between two different bacteria caused similar symptoms in humans, gram staining has become a

method to categorize bacteria based on their structure. Gram positive cells appear red and gram negative cell appear as pink.

The structural different which results in this change in color can be found in the



peptidoglycan layer. Composed of a carbohydrate polymer network with proteins attached, this layer may account for 50-90% of the cell wall in gram positive bacteria but only 10% of the cell wall for gram negative cells. Furthermore, gram-negative bacteria have an additional outer membrane.

Bacteria shape is also useful for classifying bacteria. There are 3 primary shapes, spirilla (spirals), bacilli (rods) and cocci (spherical). In this lab activity, only bacilli and cocci bacteria will be used as spirilla tend to be more pathogenic then the rest. Also, the primary shapes list previously may congregate into colonies which may aid in further classification.

Mechanism of Gram Staining

Crystal violet (CV) dissociates in aqueous solutions into CV⁺ and chloride (Cl -) ions. These ions penetrate through the cell wall and cell membrane of both Grampositive and Gram-negative cells. The CV⁺ ion interacts with negatively charged components of bacterial cells and stains the cells purple. Iodine $(I - or I_3)$ interacts with CV^+ and forms large complexes of crystal violet and iodine (CV - I) within the inner and outer layers of the cell. When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A Gram-negative cell will lose its outer membrane and the peptidoglycan layer is left exposed. The CV - I complexes are washed from the Gram-negative cell along with the outer membrane. In contrast, a Gram-positive cell becomes dehydrated from an ethanol treatment. The large CV - I complexes become trapped within the Gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both Gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds). After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged safranin or basic fuchsin, is applied last to give decolorized Gramnegative bacteria a pink or red color.



Medical Applications

For clinicians, gram staining is extremely useful in identify infections. In a clinical setting, body fluids or biopsy specimens are taken and then subjected to staining (see links below for examples). As a general rule, gram-negative bacteria are more pathogenic.

Pre-lab Activity: Microscope Tutorial

Although a familiarity with the microscope is encouraged, it is not required for this activity. Tutors will be present to help, but teachers are strongly encouraged to use the microscope tutorial (see reference below) in order to familiarize students with this tool. Additional information including quizzes, how microscopes work and a timeline outlining the development of the microscope can also be found in the reference section.

Post Lab Questions and Activities

- 1) What are the two primary components of the peptidoglycan layer?
 - Sugar and protein
- 2) Draw and label a gram-positive and gram-positive cell to scale.
 - See images above. However structures should include the following
 - o Gram +
 - Thick peptidoglycan layer
 - o Gram
 - Thin peptidoglycan layer
 - outer membrane
- 3) Which cell (gram-positive or gram-negative) shows the color of the COUNTER stain? What is the purpose of this counter stain?
 - Gram-, the counter stain makes the cells visible. Without it, they would appear clear and hard to detect with the microscope
- 4) Why is staining required to tell if a cell is gram+ or gram-
 - Cells may have the same morphology but may produce very different symptoms in the body. Gram staining provides another way to classify and therefore identify bacteria
- 5) Label and draw the 3 types of bacterial morphology.
 - Bacillus (rods)
 - Coccus (sphere)
 - Spirilla (spirals)
- 6) How do is magnification of a microscope calculated
 - Objective power multiplied by the eye piece power
- 7) When using a high powered objective, only the fine focus knob should be used, why?
 - Using the course knobs would crash the stage into the objective damaging both
- 8) What is the clinical value of the gram staining protocol
 - It will allow the physician to take a sample form the patient

References Links

Microscope Tutorial: Virtual microscope and tutor used to introduce and reinforce good microscope techniques.

(http://www.udel.edu/biology/ketcham/microscope/scope.html)

Virtual Bacteria Identification Lab: A virtual lab in which students isolate and analyze DNA from different bacteria for the purposes of identification

(http://www.hhmi.org/biointeractive/vlabs/bacterial_id/index.html)

MicrobeWiki

(http://microbewiki.kenyon.edu/index.php/MicrobeWiki)

Online Microscope Quiz (http://www.biologycorner.com/microquiz/)

History of the Microscope (http://inventors.about.com/od/mstartinventions/a/microscope.htm) (http://www.xtimeline.com/timeline/title-7)

Gram Staining Kits

(http://www.hometrainingtools.com/product_categories/31-life-science-biology-microscopiclife/products/3253-gram-stain-kit)

Medical/Clinical Application of Gram Staining

Gram Staining of Skin Lesion (http://www.ucsfhealth.org/adult/adam/data/003760.html)

Pericardial Gram Staining

(http://health.nytimes.com/health/guides/test/pericardial-fluid-gram-stain/overview.html)

Section 3: Catalase Activity

Activity Outline

- Enzymatic activity
- Catalase positive cells convert hydrogen peroxide to oxygen



Introduction for The Teacher: Catalase Activity

Catalase was first proposed to exist by Louis Jacques Thenard in the early 19th century. Thenard, the discoverer of hydrogen peroxide proposed that cells have a means to deal with this harmful product of cellular metabolism. In the early 20th century, the enzyme "catalase" was isolated and identified by Oscar Loew.

The use of catalase activity to classify a cell is slightly different then previous methods used. Where as gram staining and shape rely on the morphology of the bacteria, catalase characterization relies on the physiological properties inherent in the cell.

Catalase Reaction

$2 \ \mathrm{H_2O_2} \rightarrow 2 \ \mathrm{H_2O} + \mathrm{O_2}$

Pre-lab Activity

There is no pre-lab activity for this section **Post-lab Activities and Question**

- 1) Draw the reaction (starting with hydrogen peroxide) that catalase is involved it. Label the states of each reactant and product.
 - $2 H_2O_2 \rightarrow 2 H_2O + O_2$ (hydrogen peroxide and water are liguids, oxygen is a gas)
- 2) What is the purpose of the catalase enzyme in cells? (HINT: Where does the hydrogen peroxide come from?)
 - Catalase removes the harmful hydrogen pexiode (a product of metabolism) from the cell
- 3) In which organelle is catalase mostly likely found in human cells?
 - The peroxisomes
- 4) Catalase like many enzymes is temperature dependent. How would you design an experiment to test the activity of this enzyme? Assuming you are using catalase isolated from human livers, at which temperature do you think the enzyme would be most efficient? Draw a graph of enzyme activity versus temperature
 - Test the catalase over a range of temperatures.
 - It should be the most efficient at 37 degrees
- 5) Catalase is an enzyme; therefore what is it made of?
 - Protein
- 6) Catalase is present in ALL animals and in all tissues. Furthermore, it has nearly identical structures. Comment on the evolution history of this enzyme, when and where did it evolve? Why is there little variation between the enzyme in all animal cells? At which point in the history of life on earth do you think it evolved? (HINT: use hemoglobin as a model). If the catalase enzyme were structurally different between animals, what type of evolution is this? Explain how it came about
 - The structure and function are conserved and probably evolved relatively early in the history of life.
 - If the structures are different, it is an example of convergent evolution. Organisms suffer from the same problem (reactive species as a function of metabolism), and need to deal with it. The method may be different but the result is the same.

References and Links

Going Gray: Blame Catalase: Article discussing the connection between catalse activity and graying of the hair

(http://dsc.discovery.com/news/2009/03/02/gray-hair.html)

Catatase: Facts, and Discussion Form: Detailed outline of relevant catalse facts including, mechanism, pathology and history

(http://www.absoluteastronomy.com/topics/Catalase#encyclopedia)

Catalase: Evil Destroyer of 0₂: Experimental outline which addresses other variables such as concentration, temperature and acidity using catalase isolated from potatoes (http://www.odec.ca/projects/2006/cheu6j2/factors.html)

Virtual Catalase Regulation Simulation

(http://www.coolschool.ca/lor/BI12/unit7/U07L04.htm)

Section 4: Antibiotic Resistance

Activity outline

- Measuring of zone of inhibition
- Classifying bacteria is "susceptible, resistant or intermediate with respect to their response to antibiotics

Introduction for the Teacher: Antibiotic Resistance

Antibiotics have been a tool used by doctors for thousands of years. Although Alexander Flemming is credited with discovering penicillin 1929, historical records hinted at the use of antibiotics by the Sumerians in 3,500 B.C.E. (this early treatment involved administering beer soup laced with snakeskin and turtle shells).

The mechanisms through which antibiotics attack and kill bacteria cells vary. A basic list can be found in the picture below. However, it is important to note that modern antibiotics



ONLY work on bacteria cells. Viruses function differently than bacteria and therefore are not susceptible to these treatments.

It is also important to note that not every bacteria responds the same way to the same a drug treatment. The effect of a single drug on bacterial growth maybe tested by place a small disk of the antibiotic onto a petri dish inoculated with a single type of bacteria. By measuring the zone of inhibition, it is possible to classy the bacteria as, susceptible, resistant or intermediate.

Pre-lab Activities

There is no pre-lab activity for this lab

Post Lab Activities and Questions

- 1. List 2 targets of an antibiotic
- 2. Neusporin contains 3 different antibiotics, what is the benefit
- 3. Why don't antibiotics work for a viral infection?

- 4. Draw a plate with 3 antibiotic disks. Describe what is happen to the bacteria in the region of each disk
 - a. Disk 1 resistant,
 - b. Disk 2 susceptible
 - c. Disk 3 intermediate
- 5. When a doctor prescribes antibiotics, it is strongly recommended that the patient take the entire dosage, even if they begin feeling better. Why is this important
- 6. When dealing with a patient that has a bacterial infection, doctors usually prescribe older antibiotics first. Newer drugs are reserved for the most serious patients, why is this necessary?
- 7.

8.

Links and References

Farmers Defend Right To Use Antibiotics

(http://www.tuftsdaily.com/features/farmers-defend-right-to-use-antibiotics-in-animals-despite-nationwide-controversy-1.1646384) New York Times: Epidemics (http://www.nytimes.com/2004/03/02/science/bacteria-run-wild-defying-antibiotics.html?fta=y)

Pre Lab Vocabulary