Title: Victims of Vibrio: a Simulated Epidemiological Investigation through Biotechnology

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Abstract:

Florida is the largest producer of oysters consumed domestically in the United States and a significant percentage are harvested in or near local waters. As a component of the local seafood economy, seasonal occurrences of *Vibrio vulnificus* and recent deaths in Bay County provide students a real-life context to study an emerging pathogen with current biotechnological methods.

As representative stakeholders in a highly charged and tragic (fictional) outbreak of Vibio-related deaths, students will investigate and identify the sources of the disease while identifying personal bias and misleading information. Utilizing methods such as an ELISA, DNA extraction, PCR amplification and electrophoresis, students will identify *V. vulnificus* as the causative agent in local seafood sources.

Rational:

The purpose of this project is to enable students to make scientific connections between their curriculum, the risk of an emerging pathogen, public health considerations, and the biotechnological methods used to assess them. The unit will include technology including an ELISA, DNA extraction, PCR, and electrophoresis. Spaced throughout the course while unified by a fictional story, concepts will have the chance to be introduced and reinforced. Student developed projects will then be created to stimulate critical thinking and encourage independent analysis.

Description of Teaching Unit:

Students will randomly be assigned roles as the various stakeholders in our simulation (including reporter, relative of septicemia victim, restaurant owner of potential contamination source, etc). As the lesson progresses, character development will continue through the use of teacher generated media reports, student research, personal interviews and eventually laboratory data. Students will identify *V. vulificus* as the causative agent via ELISA testing, determine the source of contamination despite conflicting information, extract DNA from oysters, amplify through PCR, and screen for the positive result with gel electrophoresis.

Media releases will be correlated with the curriculum. For example, osmosis and hypertonic solutions will be reinforced with a news alert regarding concerning symptoms of diarrhea and water loss in our hypothetical patients. By the time students are covering the immune system in the course they will have been able to unite the common theme among most of the victims (alcoholism, diabetes, transplant recipient, etc). Computer simulations and 'dry lab' comparisons will be incorporated when possible to augment student learning. Potential guest speakers from the Bay County Health Department and the University of Florida will be pursued, as well as a possible student trip to the Oyster Industry Lab in Apalachicola. An in-character roundtable discussion will help wrap up the conclusion developed by the students. Extension projects developed by the student will help to reinforce causation and correlation: students must consider whether the disproportionate number of males can be accounted for simply because men might eat more raw oysters than women.

Expected Outcomes:

When this unit is complete, students will:

1. accurately use aseptic technique

2. develop proper pipetting skills
3. create an ELISA to screen for various pathogens
4. extract DNA from various sources
5. run a PCR to establish a functional product to measure
6. set up and run gel electrophoresis
7. perform BLAST searches to compare data
8. process epidemiological data from a variety of sources
9. eliminate distracting or misleading information through critical analysis (disproportionate male deaths, cutaneous infection from exposure to water, etc)
10. defend their discoveries from opposing viewpoints
11. understand the process and significance of keeping an accurate scientific journal
12. demonstrate learning through pre and post tests as it relates to biotechnology

Data Collection and Student Assessment:

Student pre and post test skills will be evaluated through a survey and KWLs to assess knowledge. Laboratory proficiency will be assessed formatively through simulations as the project is geared towards the inquiry process. Students will have to rely on each other to accumulate all of the information required so there will be a focus on collaboration. Final project grades will include the students' journal and oral defense of their work.

ICORE Elements Included:

Viral Quest Curriculum – Dr. Troy Sadler (UF)
Multimedia and Web-based Learning Tools - Dr. Charles D. Lawrence (UF)
UF CPET Equipment Lockers

1. Pipetting Stations (9 P-20 pipets, 9 P-200 pipets, 1 P-1000 pipet (for instructor use), Pipet tips)
2. Introduction to Gel Electrophoresis (8 horizontal gel boxes, casting trays, and combs, 2 power supplies, 8 white light boxes)
3. Advanced Gel Electrophoresis (8 E-gel Units, 1 Safe Imager, 2 power strips)
4. Thermal Cycler (PCR equipment and reagents)

Other UF Provided Resources:

5. Heat Blocks, microtube racks, microcentrifuge and minicentrifuge, vortex, microarray plates

Classroom extension activity with UF ICORE facilitators

MATERIALS: As needed for one class of 27 students working in groups of 3 – 5:

6. 3 Digital balances, test tubes, and associated glassware
7. Student gloves, parafilm, ice buckets, thermometer
8. Elisa Plates, reagents, acids, bases, and phenalphthalein
9. Oyster specimens obtained from: local restaurant, local seafood supplier, and environmental samples
10. DNA extraction lysis solutions, washes, buffers, lysing solution, tubes, dH_2O, alcohol, and splints.
11. *V. vulfnificus* primers based on environmental variant type A (vPvMH10003 -12), appropriate master mix solutions
12. Loading dye and appropriate markers, camera for gel image capture.
13. Live cultures of nonpathogenic *Vibrio fischeri* and Viral Quest classroom materials as secondary resources.

Proposed Budget:

Item	Ordering Info	Cost
Microarray Plates	Fisher Scientific: Item 21-377-203	$191.17/50
Bulk 2.0 mL Eppendorf Centrifuge Tubes	Fisher Scientific: Item 02-681-258	$23.83/500
Live culture of *Vibrio fischeri*	Science Stuff: Item BLB1038	$40
Estimated Shipping (of live cultures)	N/A	$50
Primers for 16srDNA extraction	Vulnificus environmental variant type A (vPvMH10003 -12)	Approx $50?
Oyster samples from restaurant, seafood supplier, and grocery store	N/A	Market Price?

Literature Cited:

1. Biosca, E, Marco-Noales, E, Amaro, C, & Alcaide, E. (1997, Feb) An Enzyme-linked Immunosorbant Assay for Detection of *Vibrio vulnificus* biotype 2: Development and Field Studies. *Applied Environmental Microbiology,* Volume 63 (2), pp 537 – 542.

2. Campbell, M & Wright, A. (2003, Dec). Real Time PCR Analysis of *Vibrio vulnificus* from Oysters. *Applied Environmental Microbiology,* Volume 69 (12), pp 7137–7144

3. Coleman, S, Melanson, D, Biosca, E, & Oliver, J. (1996, April). Detection of *Vibrio vulnificus* Biotypes 1 and 2 in Eels and Oysters by PCR Amplification. *Applied and Environmental Microbiology,* Volume 62 (4), pp 1378 – 1382

4. Hoffman, L. The Oyster Bacterial Biome: Who Else Is Inside the Shell? www.epicentre.com Volume 11 (5).

5. Parker, R & Lewis, D. (1995). Sandwich enzyme-linked immunosorbent assay for *Vibrio vulnificus* hemolysin to detect V. vulnificus in environmental specimens. *Applied Environmental Microbiology*, Volume 61 (2): pp 476 – 480.

6. Smith, B, & Oliver, J. (2006, March). In Situ and In Vitro Gene Expression by *Vibrio vulnificus* during Entry into, Persistance within, and Resuscitation from the Viable but Nonculturable State. *Applied Environmental Microbiology,* Volume 72 (2): pp1445 – 1451.

7. WJHG (updated: 9:26pm April 7, 2011) Bay County Health Dept: Bacteria Found in Raw Oysters Caused Death. www.wjhg.com

8. Vibrio Fact Sheet (FDA)
   http://www.fda.gov/Food/ResourcesForYou/HealthEducators/ucm085365.htm

9. Microbe Wiki: http://microbewiki.kenyon.edu/index.php/Vibrio_vulnificus

10. Infection from Consumption of Raw Shellfish or Marine-Related Wounds http://safeoysters.org/

11. Medscape Reference: http://emedicine.medscape.com/article/232038-overview

12. Interstate Shellfish State Conference: http://www.issc.org/education/vibriovulnificus.aspx

Victims' of Vibrio: a Simulated Epidemiological Investigation through Biotechnology

SUBJECT and GRADE LEVEL: Human Biology, Advanced Level (Dual Enrolled)

SCIENCE CONCEPTS: Epidemiology, genetic isolation, sequencing, human diseases, and basic molecular techniques

TIMEFRAME: While adaptable to most lesson schedules, the complete unit will encompass several weeks of background information (in the form of focus lessons at the first 10 minutes of class). Lab work and student identification are scheduled as part of a 90 block.

- Epidemiology background: focus lessons one month prior to lab work
- Pipetting Fundamentals – 1 day
- Elisa Simulation – 1 day
- DNA extraction – 1 day
- PCR amplification - 2 days
- Gel electrophoresis – 1 day
- Roundtable Assessment - 1 day

LEARNING STYLES: Students will utilize hands on lab skills as part of a kinesthetic experience to enrich their knowledge. Performances in character during the investigation and roundtable will encourage creative and original thinking skills, as well as problem solving abilities.

VOCABULARY:

Cellulitis	Gastroenteritis	Prokaryote
Filter feeder	Halophilic	Pathogenic
Food borne illness	Hemochromatosis	*Vibrio vulnificus*
Fulminant	Immune suppressed	*Vibrio cholera*

LESSON SUMMARY: Students will be presented with both real and fictional case studies that will eventually be isolated as *Vibrio* infections as part of an epidemiological simulation. Through laboratory work, students will identify, isolate, digest, and confirm the presence of *Vibrio* in local commercially harvested oysters. Simulations will be incorporated as a method of reinforcement or backup in the event of discrepant data.

STUDENT LEARNING OBJECTIVES:

As written in the affiliation agreement with Gulf Coast State College, the student will be able to...

1. Explain how the processes of metabolism that occur within living beings are chemical reactions that involve the same principals as all chemical reactions (Standard 5).
2. Describe the structure of DNA (Standard 22).
3. Define mutation, clone, and recombinant DNA (Standard 26).
4. Explain the use of genetic engineering in medicine and agriculture (Standard 27).
5. Describe the structure and function of the digestive system: mouth, esophagus, stomach, duodenum, small intestine, large intestine, liver, pancreas, gall bladder, rectum and anus (Standard 55).

MATERIALS: As needed for one class of 27 students working in groups of 3 – 5:

1. Pipetting Stations (9 P-20 pipets, 9 P-200 pipets, 1 P-1000 pipet (for instructor use), Pipet tips)

2. Introduction to Gel Electrophoresis (8 horizontal gel boxes, casting trays, and combs, 2 power supplies, 8 white light boxes)
3. Advanced Gel Electrophoresis (8 E-gel Units, 1 Safe Imager, 2 power strips)
4. 1 Thermal Cycler (PCR tubes, equipment and reagents)
5. 1 Heat Block, 3 microtube racks, 3 microcentrifuge and 3 minicentrifuge, and 3 vortex
6. 3 Digital balances, test tubes, and associated glassware
7. Student gloves, parafilm, ice buckets, thermometer
8. Elisa Plates, reagents, acids, bases, and phenalphthalein (Simulation to run from protocol from Dr. Chuck Lawrence and Drew Joseph)
9. Oyster specimens obtained from: local restaurant, local seafood supplier, and environmental samples
10. DNA extraction lysis solutions, washes, buffers, lysing solution, tubes, dH_2O, alcohol, and splints.
11. *V. vulfnificus* primers based on environmental variant type A (vPvMH10003 -12), appropriate master mix solutions
12. Loading dye and appropriate markers, camera for gel image capture.
13. Live cultures of nonpathogenic *Vibrio fischeri* and Viral Quest classroom materials as secondary resources.

Note: Items 1 – 5 available in UF CPET equipment lockers

BACKGROUND INFORMATION:

Many people fall prey each year to food poisoning. Often misunderstood or misdiagnosed, **food borne illnesses** are those diseases which infect us through our digestive system. **Pathogenic** bacteria are the common cause as they make us ill; however most bacteria (members of the Kingdom **Prokaryote**) are either harmless or actually beneficial. For many visitors to the Gulf Coast, raw oysters are a delicacy, for many locals they are savored on a weekly basis. As **filter feeders**, oysters pull water in through their tissues, absorbing anything that might also be in the water. Unfortunately, that often includes bacteria – the deadly kind.

Raw or undercooked oysters may contain ***Vibrio vulnificus***, a natural marine inhabitant that likes warm water (above 18° C). As **halophilc** organisms, the bacteria also like salty water: between 15 – 25 ppt (although salinities above 25 ppt actually have adverse effects). Vibrio has 12 primary pathogenic strains, with three causing the vast majority of deaths in the US. While ***Vibrio cholera*** (Cholera) is a frightening, well known illness, it is usually only contracted through exposure to contamination while traveling abroad. In the United States, *V. vulnificus* is the leading cause of seafood associated deaths. The CDC estimates 207 cases of *Vibrio vulnificus* in 2011, although this number is thought to be under reported as fecal scans do not often look for *V. vulnificus*.

Bacteria are taken in by filter feeders such as oysters, scallops, mussels, and clams, and during warm months the bacterial load can be as high as 1×10^6 bacteria/gram. If healthy people become infected, they may experience mild gastroenteritis (nausea, stomach pain, vomiting, and/or diarrhea) after eating raw shellfish or acquire **cellulitis** (spreading skin infection) from exposing a wound to seawater. The infectious dose of *V. vulnificus* is unknown; however **immune suppressed** individuals (those with weakened immune systems due to illness, chemotherapy, or other treatments) are at a significantly increased risk. The bacteria is iron dependent, which means those with liver disease, **hemochromatosis** (excessive iron levels), alcoholism, or related disorders are 80 times more likely to develop a case of **fulminant gastroenteritis**: a rapid, intense infection of the digestive system. The infection and severity of symptoms usually progresses very rapidly in these people. Death may occur in as few as 24 to 48 hours. In some cases, amputation of limbs is necessary to prevent death. Sadly, a previous study of North Florida indicated that less than 15% of high risk patients were aware of their increased risks with the consumption of raw or undercooked shellfish.

ADVANCE PREPARATION: Teachers should develop a 'back-story' or simulated case history that relates to their region (including local landmarks, etc). In preparation for the lab work, students should develop a confirmed diagnosis of *V. vulnificus* infection through epidemiological methods as revealed by the teacher in focus lessons throughout the unit. Alternatively, prior knowledge could be condensed into a compacted, one or two day CSI type investigation. Deliberately misleading information may be included to force students to verify their work. Prior to the bench work, students should become familiar with the *basic* science behind the molecular techniques they will be using: ELISA, DNA extractions, PCR, and gel electrophoresis. Order *V. vulnificus* primers based on environmental variant type A (vPvMH10003 -12) to isolate bacterial DNA. Immediately prior to the bench work, obtain oysters from a local restaurant, a local seafood supplier, frozen shucked oysters (from the grocery store) and environmental samples from both "open" oyster waters and "closed" (unsafe) areas if possible.

PROCEDURE

Epidemiology Background

1 day to 6 weeks: Provide students with information about recent deaths in your area. Create hypothetical victims and ask students to solve the case. As you provide students with a daily 'news alert" (focus lesson), provide just enough information to stimulate their ongoing interest in this case. Are all of the victims from the same area? Did they all have diseases in common? Are they predominantly of one race, gender or ethnicity? Information can be tied to the non-biotechnology related standards as relevant, for example a focus lesson on the physiology of Cholera deaths could precede the lesson relating to osmosis and cellular water pressure. As the epidemiologists, students should be stimulated to determine what questions they need to ask to find common factors among the victims. As students ask the thought provoking questions that lead them to their ultimate diagnosis, reveal conflicting data sets that force them to evaluate their hypothesis and examine other data. For example*, V. vulnificus* infections are found primarily (85%) in males, so one outbreak could include the illness reported from a sorority banquet (85 females attended, 29 males, yet only 4 females became sick while 11 males became sick – a disproportionate amount.) If students correctly identify the raw oysters offered at the buffet table as the culprit they are ready to progress to the next phase (otherwise, more information can be provided to lead them to their diagnosis). Assign roles as various stakeholders including: local fisherman, seafood provider, restaurant owner, gastroenteritis survivor, family member of septicemia and gastroenteritis victims, local doctor, pathologist, and journalist. **Formative assessment** will include KWLs, exit tickets, and Think-Pair-Share methods to diagnose student misconceptions. **Summative assessment** will be incorporated into previously scheduled unit tests and a diagnostic test prior to component 2.

Bench Work (8 days, 90 minute blocks)

Day 1: Pipetting Basics and Aseptic Technique: Show students how to read and calibrate a pipette of various sizes. Demonstrate the proper use and disposal of tips, and reinforce aseptic technique by modeling the right way and the wrong way to pipette. Give students a set of instructions, a 96 well plate and have them practice the use of micropipetting to accurately deliver colored solutions to prescribed wells. Proficiency will be determined through teacher observation (qualitative or **formative assessment**) and a recognizable design in the microplate (quantitative or **summative assessment**).

Day 2: ELISA/Microarray Simulation: Use the ELISA simulation (power point) developed by Dr. Lawrence to introduce the concept of a sandwich enzyme linked immunosorbent assay with students at the beginning of the period. Then allow students to "test" their various pathogens to evaluate their hypothesis (developed from their epidemiology background). Students will use a microarray test with hypothetical antibodies (in reality, phenalphthaleine laced bits of paper). The *V. vulnificus* samples will test positive (as they are really a weak NaOH or other basic solution). Students should periodically be asked to justify the reason for what they are measuring (**formative assessment**) and provided clarification when needed. Reflections and correct protocols in their lab journals will later be assessed as **summative measurements**.

Day 3: DNA extraction: Begin by reviewing the basic properties of DNA through a talk-aloud, and ask students to identify cellular layers or chemical that must be broken to release DNA into solution. Using universal safety precautions remove oysters from ice and blend. Distribute samples to students' lab stations where they can add a lysis buffer (soap), binding solution (enzymes), and wash solutions (alcohol) to separate the whole DNA from the tissue. At this point you have the DNA from the oyster and any microorganisms that are present. Alternatively, students can grind up oysters in solution and grow up microbes for 4 to 24 hours, freeze, lyse open the cells and proceed to PCR analysis. **Formative assessment** will be measured by the presence of a DNA sample and through teacher observation. Students will also fill out an exit ticket to reinforce the reason for the procedure at this point in the process. **Summative assessment** will be reflected in their lab journal.

Day 4 – 5: Polymerase Chain Reaction: Develop student interest at the beginning of class by showing the PCR Song (YouTube – via Biorad). Review the basic denature-annealing-extending protocol that will allow for the primers to separate *V. vulnificus* from other DNA. Model correct procedure and guide students in the master mix preparation. Run the appropriate cycle to amplify the cut *V. vulnificus* DNA. Collect samples and then allow students to proceed to verification. A pre and post test will provide for **formative assessment** and a webquest PCR lab from learn.genetics.utah.edu will be used for **summative measurement.**

Day 6(ish): Gel Electrophoresis: Introduce the concept through footballphoresis, an activity completed outdoors for the first 10 minutes of class where students represent the various sizes of DNA (individually, in small groups, and in one large group) as they RUN down the gel (the open field) based on the charge (the teacher's cue). Proper understanding of the components illustrates **formative assessment.** Then add the previous day's PCR product with a loading dye and markers to appropriate lanes of an E gel and (cut run. Capture image within class if time permits or document and hold for the follow up. A single band will confirm that the primers bound and amplified *V. vulnificus* (positive) and no bands will indicate a negative result. Regardless of results, student **summative assessment** will be based on proper recording technique and justification for their conclusions.

Wrap Up and Extension: Following lab work, students will present a summary of their data, lesson and experiences at a roundtable in the character they were assigned earlier. Students will develop an educational outreach program to educate the high risk consumers in our area (ideas will be prompted by the mp3 "Fifty Ways to Eat Your Oyster" sung to the tune of "Fifty Ways to Leave Your Lover" by Paul Simon).

ASSESSMENT: Pre and post tests will be developed and used for each major component, in addition KWLs, exit tickets, and other informal assessments will be used as formative feedback throughout the process. Formal evaluation will be based on their oral presentation at the round table and their written lab journals.
Written lab reports will demonstrate student proficiency in collecting and organizing qualitative and quantitative data. Reports will also demonstrate students' ability to analyze results and form logical conclusions based on data analysis.

Literature Cited:

1. Biosca, E, Marco-Noales, E, Amaro, C, & Alcaide, E. (1997, Feb) An Enzyme-linked Immunosorbant Assay for Detection of *Vibrio vulnificus* biotype 2: Development and Field Studies. *Applied Environmental Microbiology,* Volume 63 (2), pp 537 – 542.

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