Building a Biomonitor:
Bean Beetle Larvae as a Model for Detecting Intestinal Bacteria Pollution in Water

Student Handout

Objectives

The overall outcome of this long-term activity is for you to develop a simple, but accurate research model for testing the presence of cell toxins in environmental samples. You will be asked to use two existing cell toxicity strategies and a proposed bean beetle research model to measure endotoxins in water accurately, inexpensively, and rapidly.

- You will design a test that determines whether bean beetle larvae are susceptible to intestinal bacteria endotoxins.
- You will design a controlled experiment to evaluate how the *Limulus* amoebocyte lysate (LAL) test can be blended with the trypan blue test of cell viability to test for endotoxins.
- You will evaluate the feasibility of using the test you develop by evaluating its accuracy, cost, and simplicity.

Introduction

Biological monitoring, or biomonitoring, makes use of organisms to provide information about environmental quality. It is proving to be a reliable way of determining the presence many types of pollutants in air, soil, and water (CDC 2013). Biomonitoring can be done in the field on populations of organisms or can be performed in a laboratory on the biochemistry of an individual organism. Scientists are discovering that biomonitoring provides some of the best data for detecting minute amounts of a pollutant and for investigating the long-term effects of a pollutant on an organism. Biomonitoring is a rapidly growing field that makes use of microorganisms, animals, and plants as biomonitors.

Background

Water pollution is a major issue worldwide and prevents many people from having access to safe water (UN 2013). Three major types of water pollution are bacteria, dirt, and nutrients (EPA 2012). In developing nations water pollution decreases the availability of clean water needed for bathing, drinking, and cooking. In developed nations, water pollution greatly contributes to the cost of maintaining clean waterways used for commerce, consumption, and recreation. A disturbingly common pollutant is intestinal bacteria from animal and human sources. Most often, water needed for human consumption is contaminated with human intestinal bacteria (CDC 2012). Unlike chemical pollutants, human bacteria pollution can cause infectious diseases that spread from person to person. Infected people can also accidentally pollute clean water supplies. Much of the harm from the bacteria is due to a variety of enzymes and toxins released as the bacteria feed and replicate.

It is possible to detect and monitor intestinal bacterial pollution in water using a variety of techniques. However, there are few inexpensive, quick, and simple procedures for

accurately determining the presence of intestinal bacteria in water (Deininger and Lee 2005). Even these procedures are not feasible in many situations where urgent water testing is needed, such as in developing nations with limited resources (Zakir Hossain et al 2012).

Problem

You have just learned that you were selected for an internship with a nonprofit group that studies global water issues. The group just formed a team that will investigate inexpensive and simple methods for determining the presence of intestinal bacteria in drinking water. This team is being led by a researcher, Dr. Erica Ojobi, who previously researched mysid shrimps as indicators or biomonitors of water pollution (Toussaint et al 1995). She did this by monitoring the health of the mysid shrimp in response to the different pollutants. Mysid shrimp are easy to grow and their response to pollutants is simple to measure. Plus, the method uses easy-to-find materials and does not need to be conducted in a laboratory.

After hearing about the natural history of bean beetles, *Callosobruchus maculatus*, at a conference, Dr. Ojobi was interested in using the beetle larvae as indicators of intestinal bacteria pollution in water supplies (Beck and Blumer 2007). She also remembered studying two procedures that could help determine the health of the beetles when exposed to intestinal bacteria pollution. One test, called the *Limulus* amoebocyte lysate (LAL) test, uses horseshoe crab blood cells as indicators of bacterial endotoxins (Lonza Group 2013, Prior 1990). Endotoxins are harmful molecules released by specific bacteria during death and replication. Certain endotoxins at very low levels are known to disrupt and sometimes lyse certain cells (Yu et al 1997, Svensson et al 2005).

Another test, called the trypan blue test, is used to investigate the health of cells (Sigma-Aldrich 2013). Trypan blue is a dye that is not taken up by healthy cells. However, trypan blue will pass through the membranes of dead and dying cells making the inside of these cells dark blue (Figure 1).

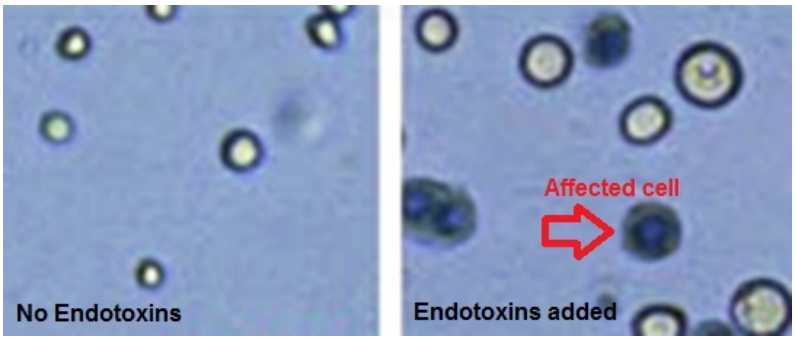

Figure 1: Trypan Blue Test Results

Dr. Ojobi has a hypothesis that the LAL tests and the trypan blue tests can be blended and modified in a way to test for intestinal bacteria in water. She is hoping to come up with a simple and low cost way to biomonitor the bacteria. Dr. Ojobi believes that bean beetles will be the ideal organism to use for developing this biomonitoring test.

Materials

Remember that Dr. Ojobi's work is focused on finding ways of monitoring pollutants using simple way and low cost strategies. Plus, she has limited funds and materials to carry out her work. Consequently, you are limited to the types of materials that are available in her work area for you to conduct your experiment.

Fortunately, Dr. Ojobi is prepared for the study and made an inventory of materials in her lab (that inventory list is available from your instructor). It is important that you be selective about the materials you use to conduct your experiment. Use only what is required to perform your specific experiment. Before requesting materials, please review the inventory list and evaluate whether each item on the list is useful for the success of your experiment. You may have the option of requesting other items by submitting a request to your instructor. In your request, you must justify why you want the item and what can be used in place of the item if it is not available. Keep in mind that you can substitute items in the inventory for less-expensive items that lower the cost of the experiment.

Experimental Design

The purpose of your experiment is to see whether bean beetle larvae can be used as biomonitors for intestinal bacteria in water. You will be using components of the LAL test and the trypan blue test to detect any effects of the endotoxins on the bean beetle larvae. In order to test Dr. Ojobi's hypothesis for this experiment, you need investigate the reasoning why she made the hypothesis. Use the references provided in this activity and the Internet to answer following questions:

Bean Beetle Questions:

1. Why did Dr. Ojobi think that bean beetles would make a good model for studying pollution?
2. What do you need to know about the bean beetle life cycle that might make them vulnerable to the endotoxins of intestinal bacteria?
3. How would endotoxins affect bean beetle cells based on what is known about other organisms?

Endotoxin Testing Questions:

1. What feature of the LAL test do think Dr. Ojobi finds valuable for indicating the presence of endotoxins?
2. What information is provided by the trypan blue test and could it be valuable in looking for the presence of endotoxins?
3. How would you combine the two tests to come up with one test that could indicate the presence of endotoxin damage to cells?

Experimental Design:

1. What role would the bean beetles larvae play in the experimental design?
2. Identify the independent variable for your experiment.
3. Identify the dependent variable for your experiment.
4. What variables need to be constant your experiment.
5. Describe the experimental design for your experiment.
6. Describe how you would recognize if the bean beetle larvae are being affected by the endotoxins.
7. How would you set up the control group for this experiment?
8. How would you set up an experiment to determine if other pollutants and other types of bacteria give the same experimental results as the endotoxins?
9. What type of data would you have to collect to determine if the bean beetle larvae are responding to the endotoxins?
10. Describe any statistical analyses you need to compare your experimental group to your control group.
11. Explain if your experimental method can meet Dr. Ojobi's criteria of being accurate, inexpensive, and simple to do.

Come to class prepared to present your experimental design.

Literature Cited

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This experiment was written by Ms. Betsy Morgan and Dr. Brian R. Shmaefsky, 2014 (www.beanbeetles.org).