

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

Instructor's Notes

Background

There is ample evidence in the science education literature that engaging students in authentic experiments enhances science content comprehension and retention (Leonard 1991, Brickman et al 2009). One caveat is that the experiments should not be cookbook style and must be student-centered in order to reinforce the principles of experimental design (McClanahan and McClanahan 2002). Unfortunately, student-centered experiments can be an overwhelming task for faculty.

One difficulty is foreseeing, and having readily available, the materials and resources needed for the students to complete their open-ended study. However, much of this concern can be remedied by limiting the types of materials the students can work with. Another problem is restricting the student investigation to a certain body of literature and limiting their lines of reasoning to a targeted goal without giving too much instructor direction. Limitations can be placed on an investigation by putting it into an informed designed setting (Crismond and Adams 2012). An informed designed scenario requires the collection of a specific body of knowledge to resolve a well-defined problem.

It is important to stress to yourself and to your students the purpose of this activity. This activity was developed to analyze how students use biological principles to resolve a problem using an informed design scientific investigation process. The specific mission for students is to use two existing technologies [LAL (Bryans et al 2004) and trypan blue (Strober 2001) assays] and a proposed research model (bean beetles) to measure endotoxins in water accurately, inexpensively, and rapidly.

Purpose and Learning Objectives

This activity shows students how research models can be used to understand factors that affect homeostasis at the cellular level. It blends two cell study technologies: *Limulus* amoebocyte lysate assay and trypan blue vitality staining. The biology topics covered are:

- Apoptosis
- Cell membrane
- Cell structure
- Cell staining (vital stains)
- Experimental method
- Programmed cell death
- Research models

The activity's level-appropriateness is determined by the degree of detail expected in the complexity of the experimental design and in the explanation in the discussion section of student reports.

- Non-majors should be expected to design a proper controlled experiment comparing cell lysis with and without endotoxins based on the materials provided. Their level of explanation of the endotoxin effects on the insects

should include that the endotoxins lyse the insect cells by disrupting the cell membrane. They should also be able to explain that trypan blue enters only damaged cells. Students should explain that damage to amoebocytes can be correlated to cells in other organism.

- Introductory majors should be expected to design a proper controlled experiment comparing cell lysis using a standard (a chemical known to cause cell lysis, a negative control, and an endotoxin). They should design the experiment based on the materials provided in addition to other relevant materials. Their explanation of the endotoxin effects on the insects should include susceptibility of the bean beetle cells to endotoxins and that endotoxins are molecules that attach to insect cells and cause organelles to release compounds that degrade the cell. Students should explain that damage to amoebocytes can be evolutionarily correlated to cells in other organism.
- Intermediate majors should be expected to design a proper controlled experiment comparing cell lysis using a standard (a chemical known to cause cell lysis, a negative control, and an endotoxin). The students should show evidence of the various environmental variables that should or should not be controlled based on how they would significantly affect the results. They should design *in vitro* and *in vivo* the experiment based on the materials provided in addition to other relevant materials. Their explanation of the endotoxin effects on the insects should include the role of natural selection in susceptibility of the bean beetle cells to endotoxins and that endotoxins are molecules that attach to insect cells and cause a degranulation event that degrades the cell. The students should recognize that not all cells are lysing; only the amoebocytes from the insect are lysing and the amoebocytes are present in early larval stages. Students should explain that damage to amoebocytes can be evolutionarily correlated to immune system cells in other organism.

Time Required to Complete this Activity

- Planning and experimental design phase (out of class) 6 hours
- Experimental phase
 - Practice removing the embryos 2 hours
 - Removing viable embryos for experiment 1/2 hour
 - Endotoxin preparation 1 hour
 - Trypan blue testing 1 hour
- Data Analysis and write-up (out of class) 6 hours

Experimental Design

The specific objective of the activity is to use bean beetles and a combination of two cytotoxicity assays to develop a research model for determining the presence of intestinal bacteria in water. This objective is set within the parameters that students will be asked to review the scientific literature on using insects as environmental pollution indicators, bacterial endotoxins, LAL testing, and trypan blue assays. In their informed designed project, students must be aware of the cost, rapidity, and simplicity of the procedure to assess its feasibility.

Students should be tackling the following questions when developing their procedure:

- How can bean beetle larvae be used as a model for environmental toxicology?
- Are bean beetle cells susceptible to bacterial endotoxins in water contaminated with intestinal bacteria?
- How can the *Limulus* lysate test and the trypan blue test be applied to study endotoxins?
- Are bean beetles an accurate model of testing the presence of environmental toxins such as endotoxins?
- Is the endotoxin test that is developed feasible for use in situations where inexpensive and rapid testing is needed?
- Can the endotoxin test that is being developed be generalized to investigations on other environmental pollutants?

As the students are planning their experiments, it is important to remind students to keep their experiments simple. Additionally, remind the students the type of study they are doing must take in the following considerations:

1. Does the control group differ only in the independent variable from the experimental group?
2. Are the results of the experiment unique to intestinal bacterial pollution or can the results be explained by any type of common water pollutant or harmless bacteria?
3. If the experiment works, can it be done easily with reasonable low cost?

One major confounding issue with data collection is the harm caused to the larvae when they are removed from the bean. Students should be asked to justify if cell damage monitored in the trypan blue test is the result of the procedure or the result of the bacterial toxins.

Provide students with Figure 2 (a – f) from Page 4 of A Handbook on Bean Beetles, *Callosobruchus maculatus* (www.beanbeetles.org/handbook) as a reference when they are removing of the larvae.

Data Collection

Students typically come up and approve the following experimental protocol for this activity:

1. Set up microscopes with digital cameras
2. Collect beetle larvae
3. Place larvae in clear saline solution
4. Clean away all bean residue
5. Tease apart larvae to expose internal cells
6. Place cells on microscope slide with water (distilled, pond, saline, or seawater) and no coverslip

7. View cells under microscope and collect images. Note that the larvae cells are spherical and the bean cells are oblong (see Figure 1)
8. Add trypan blue to slide and collect images
 - a. Viable cells are clear or have background color
 - b. Dead or disrupted cells are dark blue (arrow)
9. Add treatment (*E. coli*, water, or pollutant) and collect images
 - a. Negative Control – water being used for study (distilled, pond, saline, or seawater)
 - b. Positive Control – disinfectants, pesticides (pyrethrins or mosquito agents), or some treatment that kills cells
 - c. Specificity – comparison to other common water pollutants such as sediment (soil), nutrient pollutants (fertilizers), and harmless bacteria.
10. Count relative number of cells disrupted or killed for each treatment
11. Record data in spreadsheet including summing and averaging the data for control and experimental groups
 - a. Data are typically recorded as number of cells killed out total count
 - b. Data are typically compared graphically and statistically as percentage of cells killed
12. Perform statistical analysis (Chi-Squared or T-test) to evaluate differences in the results

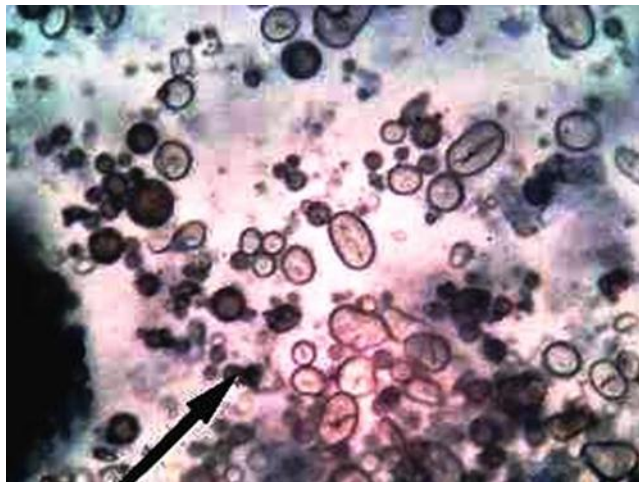


Figure 1. Experimental results of *E. coli* exposure to bean beetle larval cells. Arrow points to a larval bean beetle cell (spherical shape) whereas the oblong cells are of the host bean.

The classes mostly agreed upon a simple experiment comparing the experimental group to a negative control. However, the class should be encouraged to set up a positive control using any chemical or condition (for example, osmolarity) that causes cell lysis. Plus, students like to see if all the bacteria made available to them cause bean beetle cell lysis. Typically, students are aware of setting constant variables between the groups. They know to watch for the following variables:

- Temperature
- Water type being used

- Fluid volumes
- Amount of bacteria exposed to the beetles
- Amount of trypan blue added to experiment
- Mass or volume of beetle larvae being tested
- The isolation or clumping of larval cells being studied
- Growth conditions of beans (regular versus organic)
- Familial generation of the bean beetles

However, students should be encouraged to recognize that the developmental stage of the larvae may make a difference in the results.

Data Analysis

By knowing the theory of the trypan blue tests, students are well-aware that they need to record their data as the percent of larval cells killed either on a field of isolated cells or cells found in small clumps. Students usually agree to collect data digitally by photographing the slides using digital cameras attached to the microscopes or by using the cameras on their cell phone through the microscope ocular. They then do cell counts from the digital images by recording the number of cells killed out total count of cells.

The data are typically compared graphically and statistically as the percentage of cells killed tallied for each group including any replicate studies. Most often students use spreadsheet graphing features to present their data. Simple data tallying is done with the spreadsheet formulas feature. However, the statistical analysis such as Chi-Squared and Student T-test are done using scientific calculators or statistics websites such as Stat Pages Org at <http://statpages.org/>. T-tests are conducted by comparing control to experimental group, for the simpler studies. Students who compare three or more groups run multiple T-tests and will compare the groups in the following way:

Variation 1

- Negative control group to experimental group
- Positive control group to experimental group
- Negative control group to positive control group

Variation 2

- Control group to *E. coli* experimental group
- Control group to *Lactobacillus* experimental group
- Control group to *Staphylococcus* experimental group
- *E. coli* experimental group to *Lactobacillus* experimental group
- *E. coli* experimental group to *Staphylococcus* experimental group

Students have never selected an analysis of variance (ANOVA) test to compare multiple data groups. ANOVA works best for comparing multiple groups, particularly for Variation 2.

Equipment and Supplies

The strength of this activity is providing a limited amount of materials that students must make use of to successfully design their experiment. The informed design model of inquiry gives students a task and specific materials to

resolve an issue. The issue in this activity is to biomonitor intestinal bacteria using a specific biomonitor organism and simple to interpret analytical methods.

Students are prompted to ask the instructor for the list of materials provided below. The items on this list are an inventory in a hypothetical laboratory where the students are interning. They must make use of only those materials needed to design the experiment according to the criteria set by the principle investigator in the study (Dr. Erica Ojobi). Students also are prompted to ask for inexpensive substitute materials. Students are instructed submit to the instructor a formal request for any items on the list knowing that that may not get their request fulfilled. Much of what they will request is likely commonly available in most teaching laboratories.

Inventory

Organisms

- Regular mung beans (*Vigna radiata*)
- Organic mung beans
- Regular mung beans containing bean beetle (*Callosobruchus maculatus*) eggs and larvae
- Organic mung beans containing bean beetle eggs and larvae
- *Escherichia coli* strains B or C broth cultures
- *Lactobacillus acidophilus* broth cultures
- *Staphylococcus epidermidis* broth cultures

Dissection Materials

- Lab scissors
- Forceps
- Straight teasing needles
- Dissecting trays

Reagents

- 0.9% (W/V) Saline solution
- Trypan Blue solution (0.4% W/V) (0.4 grams of trypan blue powder to 100ml of distilled water)
- Methylene blue solution
- Laboratory disinfectant solutions
- Distilled water
- Pond water mixture (1.0% W/V NaCl) (1.0 gram of sodium chloride to 100ml of distilled water)
- Seawater mixture (3.5% W/V NaCl) (3.5 grams of sodium chloride to 100ml of distilled water)
- Liquid houseplant fertilizer
- Pyrethrin based pesticide
- Mosquito Dunks[®] or Mosquito Bits[®]

Miscellaneous Materials

- Petri dishes (35mm)
- Well plates – 96 count
- Plastic lab droppers (pipettes)
- Test tubes with racks
- Microbiology transfer loops
- Bunsen burners
- Microscope slides
- Microscope slide coverslips
- Cell counting chambers
- Filter paper, 35 mm # 1
- Local soil sample

Laboratory Equipment

- Transmission light microscope
- Dissecting microscope or hand lens
- Digital camera for microscope
- Adjustable water bath
- Bench top balances
- Incubator
- Heating plates
- 50 ml graduated cylinders
- 100 ml beakers
- 250 ml beakers
- 500 ml beakers
- 250 ml Erlenmeyer flasks

Personal Protection Equipment

- Lab gloves
- Safety glasses or goggles
- Lab apron or lab coat

Literature Cited

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