The Dengue Dilemma
Lesson Three Adapated from BioRad

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The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

Additional information regarding the Bench to Bedside project is available at http://www.cpet.ufl.edu/bench.

Please direct inquiries to Julie Bokor at jbokor@ufl.edu or 352.392.2310.

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Introduction

Admittedly, I find pathogens intriguing. In particular, dengue is one of the more interesting. Here is this tiny RNA virus that has four different forms, and can either cause minor discomfort or trigger excruciating pain and even death. It does not exist on its own, but is dependent on a female mosquito to foster its life until its virion daughters make their way into a human host where it multiplies, destroying human cells and body systems. This is a disease we can control quite easily if we can break the life cycle of just two species of mosquito. Preventing these mosquitoes from breeding or getting rid of mosquito larva to halt future generations, and dengue can no longer be transmitted. In developing countries, this is a much harder task than in ours with air conditioning, window screens, indoor living and excellent water and sanitation systems. However, even in our country, we see outbreaks of dengue fever, mostly along the Texas/Mexico border. The Southeastern United States is home to the species of mosquitoes that carry and transmit dengue virus so the very real possibility exists that once introduced, dengue could become endemic in our country. In 2009 and 2010 this reality struck Key West and south Florida.

Author’s Note

In this unit, students will follow the initial case of the dengue outbreak in Key West, Florida in 2009. Nestled between the narratives will be the opportunity for the students to perform clinical tests and take on the role of the diagnostic laboratories. They will also go out into the field, and perform mosquito surveys to see which species are present, where, and relative abundance in an effort to determine the source of the dengue mosquito, and suggest control measures to prevent further spread of the disease.

This particular topic was chosen as a result of a grant from the Howard Hughes Medical Institute to the UF Center for Precollegiate Education and Training. As part of a two week institute, Dr. Roxanne Connelly, an entomologist located at UF’s Florida Medical Entomology Laboratory, has devoted an entire day to our teachers for the past six years and served as a mentor to some as they implement new lessons in their classrooms. Dr. Connelly discusses arthropod vectors in general, and mosquitoes and mosquito borne diseases in particular. The dengue outbreak during 2009 and 2010 was unfortunate for those affected, but served as an incredible teachable moment for our program. Coincidentally, the 2010 HHMI holiday lecture series focused on dengue fever, providing another fantastic resource for teaching about many topics including molecular biology, epidemiology, and virology. With additional funding from the National Institutes of Health Science Education Partnership Award, dengue provides an excellent opportunity to illustrate the interaction between humans and the environment, the impact those actions can have on the health of an entire community, as well as the medical mystery of dengue and the immune response to the different serotypes. Looking at translational research, there is much work devoted to developing a vaccine, with clinical trials underway evaluating the efficacy and safety of different formulations. Always trying to minimize harm, is it possible to vaccinate against dengue without then putting a vaccinated person at risk for subsequent infection and increased immune response?
Tips About this Curriculum

Lesson Plan Format: All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

KEY QUESTION(S): Identifies key questions the lesson will explore.

OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

STANDARDS: Specific state benchmarks addressed in the lesson. Also collected in one list. This curriculum is also aligned with common core and Next Generation Science Standards (NGSS)

MATERIALS: Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.

ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.

PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Teachers should feel free to create additional formative and summative assessment pieces.

EXTENSIONS (ACTIVITIES/LITERATURE): There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available.

STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.

TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.
Collaborative Learning: The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in teacher-driven, lecture format, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

Groups: Most of the lessons are carried out in groups. While it isn’t necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

Inquiry-based: The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

Technology: Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

Content: This unit provides an opportunity to synthesize discrete content facts into an authentic context. Students take concepts learned such as immune response and clinical testing procedures, and put them in the context of disease. The lessons aren’t designed to teach students the intricate details of the immune system or determining an index case in an outbreak, but rather why these ideas are important and how researchers can use them.

Implementation Notes: This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

Extensions: There are many opportunities to expand the lessons presented here. To help students understand the importance of vector control and mosquito biology, a concurrent activity of rearing mosquitoes can help the students understand the life cycle of the vector host and its prevalence in the environment. Additionally, you may wish to expand on the idea of vaccine development, both the difficulties related to dengue and the history and current controversy over vaccination in the United States. Service projects would be a natural extension, particularly in areas with large mosquito populations.

Science Subject: Biology

Grade and Ability Level: 9-12 students in all levels of biology

Science Concepts: virus, disease transmission, vectors, antibodies, antigens, DNA, proteins, replication, immune response
Lesson Summaries

LEsson ONE: What Ails You?
Students will use the first case report from the Key West 2009 dengue outbreak to complete an epidemiological report. This lesson begins with her initial symptoms and visit to her primary care physician. Students will return to this epidemiological report as the case develops through the lessons.

LEsson TWO: Steps of an ELISA
Student match diagrams with text descriptions to understand the steps of an ELISA. A common test used to detect if a patient has been exposed to dengue virus is called an ELISA (enzyme linked immunosorbant assay). This test takes advantage of the interactions between antigens and antibodies. Often compared to a lock and key, an antigen/antibody interaction is very specific.

LEsson THREE: Testing for Dengue Antibodies
Using a commercial, classroom-friendly ELISA kit, students will test the patient serum sample for the presence of dengue antibodies, and record their results on the epidemiological report. A simulated version is also presented.

LEsson FOUR: Gel Electrophoresis
Using simulated PCR products, students will perform gel electrophoresis to determine which serotype of dengue virus our patient is infected with. They will have positive controls for all four serotypes and compare them with the patient’s cerebral spinal fluid sample taken early in the course of her infection. The students will determine that our patient is positive for serotype 1 (DENV1) and record this information on their epidemiological report.

LEsson FIVE: Different Tests for Different Stages
Different assays are used to test for and diagnose dengue virus. The two main tests utilized are the ELISA and RT-PCR. Students have now learned about each of these assays and should consider why each test was performed depending on the sample and date taken. Using the host response graph, students will answer questions to help clarify their thinking and then apply this knowledge to patient case #1.
Lesson Sequencing Guide

Since the classroom teacher knows his or her students best, the sequencing of lessons and the amount of time spent on each should be altered to meet the needs of each individual setting. Below is a suggested pacing guide that can be used when planning to use this curriculum, assuming 45-minute class periods.

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesson 1: What Ails You? (45 minutes)</td>
<td>Lesson 2: Steps of an ELISA (45 minutes)</td>
<td>Lesson 3: Testing for Dengue Antibodies (45 minutes)</td>
<td>Lesson 4: Gel Electrophoresis (45 minutes)</td>
<td>Lesson 5: Different Tests for Different Stages (45 minutes)</td>
</tr>
</tbody>
</table>
Vocabulary

Acute: sudden onset of disease; short duration

Agarose: derivation of agar used as a medium for gel electrophoresis

Antibody: protein produced by B cells in response to an antigen to neutralize the foreign protein (antigen); also called immunoglobulin.

Antigen: any substance that is foreign to the body and stimulates an immune response

cDNA: complimentary DNA; made via reverse transcription from a mRNA strand

Dengue: an acute infectious disease that is characterized by headache, severe joint pain, and a rash and that is caused by a single-stranded RNA virus of the genus Flavivirus (species Dengue virus) transmitted by mosquitoes of the genus Aedes—called also breakbone fever. (From the Merriam Webster dictionary.)

ELISA: Enzyme Linked Immuno Sorbant Assay – antigen/antibody assay

Endemic: restricted to a particular location or region

Epidemiology: a branch of medical science that deals with the incidence, distribution, and control of disease in a population

Gel Electrophoresis: separating DNA or proteins by size through a matrix by applying an electrical current

Polymerase Chain Reaction: in vitro synthesis of a specific portion of a DNA molecule through a cycling of three steps: denaturation, annealing, and extension

Primary Antibody: in ELISA, the first antibody bound

RT-PCR: reverse transcription PCR. Using an RNA template to create a complimentary DNA (cDNA) strand that can then be amplified via polymerase chain reaction

Secondary Antibody: in ELISA, the second antibody bound, increasing the sensitivity of the assay

Viral Load: quantitative measure of virus present in a biological system

Viremia: presence of viruses in the blood
### Next Generation Sunshine State Standards – Science

<table>
<thead>
<tr>
<th>Standard</th>
<th>Lesson</th>
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</thead>
<tbody>
<tr>
<td>SC.912.L.14.52</td>
<td>1</td>
</tr>
<tr>
<td>Explain the basic functions of the human immune system, including specific/nonspecific immune response, vaccines, and antibiotics.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.16.10</td>
<td>1</td>
</tr>
<tr>
<td>Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.16.11</td>
<td>1</td>
</tr>
<tr>
<td>Discuss the technologies associated with forensic medicine and DNA identification, including RFLP analysis.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.16.12</td>
<td>1</td>
</tr>
<tr>
<td>Describe how basic DNA technology (gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.L.18.1</td>
<td>1</td>
</tr>
<tr>
<td>Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.N.1.6</td>
<td>1</td>
</tr>
<tr>
<td>Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.N.3.5</td>
<td>1</td>
</tr>
<tr>
<td>Describe the function of models in science, and identify the wide range of models used in science.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.N.4.1</td>
<td>1</td>
</tr>
<tr>
<td>Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society’s decision making.</td>
<td>X</td>
</tr>
<tr>
<td>SC.912.N.4.2</td>
<td>1</td>
</tr>
<tr>
<td>Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.</td>
<td>X</td>
</tr>
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</table>
Background Information:

Dengue fever is an infectious tropical disease caused by the dengue virus. Symptoms include fever, headache, muscle and joint pains, and a characteristic skin rash that is similar to measles. In a small proportion of cases the disease develops into the life-threatening dengue hemorrhagic fever (also called severe dengue), resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs.

Dengue is transmitted by species of mosquito within the genus Aedes, principally Ae. Aegypti (the yellow fever mosquito). Ae. albopictus (the Asian tiger mosquito) has also proven to be a competent vector.

The incidence of dengue has increased 30-fold over the last 50 years. Up to 50-100 million infections are now estimated to occur annually in over 100 endemic countries, putting almost half of the world's population at risk. Apart from eliminating the mosquitoes, work is ongoing to develop a vaccine, as well as medication targeted directly at the virus.

The Virus

The dengue virus (DENV) comprises four distinct, but closely related, serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) which belong to the genus Flavivirus, family Flaviviridae. All four serotypes cause dengue fever. Distinct genotypes have been identified within each serotype, highlighting the extensive genetic variability of the dengue serotypes. This is also important research, as some genotypes are indicated with varying disease severity. For instance, “Asian” genotypes of DENV-2 and DENV-3 are frequently associated with severe disease accompanying secondary dengue infections.

Recovery from infection by one dengue virus provides lifelong immunity against that particular virus serotype. However, this immunity confers only partial and transient protection against subsequent infection by the other three serotypes of the virus. Evidence points to the fact that sequential infection increases the risk of developing severe dengue. The time interval between infections and the particular viral sequence of infections may also be of importance.

Transmission

The Aedes aegypti mosquito is the primary vector of dengue. The virus is transmitted to humans through the bites of infected female mosquitoes. After virus incubation for 4–10 days in the mosquito mid-gut, an infected mosquito is capable of transmitting the virus through its salivary glands during feeding for the rest of its life.

Infected humans are the main carriers and multipliers of the virus. Patients who are infected with the dengue virus can transmit the infection via Aedes mosquitoes for 5-12 days after their first symptoms appear.

Aedes aegypti adults are found within or near human environments, often biting indoors (in places without extensive air conditioning use) or in sheltered areas near houses. This mosquito is predominantly a day biter, but may rarely bite early in the night. Female Ae. aegypti bite multiple people during each feeding period, so an infected mosquito can quickly spread disease throughout a community. Containers of water, both natural and artificial, serve as larval habitats for this species. Examples include discarded cans, tires, roof gutters, water barrels, flower pots, phytotelmata (plant held water bodies such as those occurring in bromeliad axils and tree holes), miscellaneous water holding debris, ponds, wetlands, retention ponds, abandoned pools, and many others.

Aedes albopictus, a secondary dengue vector from Asia, has spread to North America and Europe largely due to the international trade in used tires (a breeding habitat) and other goods (e.g. lucky bamboo). Ae. albopictus is highly adaptive and therefore can survive in cooler temperate regions as well. Its spread is due to its tolerance to temperatures below freezing, hibernation, and ability to shelter in microhabitats.
Characteristics

Dengue fever is a severe, flu-like illness that affects all ages, but seldom causes death.

Dengue should be suspected when a high fever (40°C/104°F) is accompanied by two of the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands or rash. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito.

Severe dengue is a potentially deadly complication due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs occur 3–7 days after the first symptoms in conjunction with a decrease in temperature (below 38°C/100°F) and include: severe abdominal pain, persistent vomiting, rapid breathing, bleeding gums, fatigue, restlessness, blood in vomit. The next 24–48 hours of the critical stage can be lethal; proper medical care is needed to avoid complications and risk of death.

Treatment

There is no specific treatment for dengue. Treatment of acute dengue is supportive, using either oral or intravenous rehydration for mild or moderate disease, and intravenous fluids and blood transfusion for more severe cases. For severe dengue, medical care by physicians and nurses experienced with the effects and progression of the disease can save lives – decreasing mortality rates from more than 20% to less than 1%. Maintenance of the patient’s body fluid volume is critical to severe dengue care.

Prevention

Prevention of dengue involves avoidance of mosquito bites, either by reducing mosquito vector populations, or by using personal protection measures such as protective clothing and repellents, and/or avoidance of mosquito infected areas. Mosquito population control involves spraying larvicides, removal of water-holding containers such as discarded tires and cans, and public water projects that improve drainage and reduce the need for household water storage. Recent successes in dengue reduction using integrated pest management techniques, including community education and biological control with copepods have been reported.

Vaccination

Due to the unique nature of dengue, immunity against all serotypes must be induced at one time. It has been difficult to develop a vaccination against all serotypes and circumvent antibody enhancement without harming subjects. In recent years, however, the development of dengue vaccines has accelerated dramatically. Today, several vaccines are in various stages of advanced development, with clinical trials currently underway on five candidate vaccines.

There are many sources of excellent information about dengue fever and the mosquito vector. Teachers may wish to view the original sources for images, detailed explanations, and as print resources for their students. The information provided here was excerpted from the following sources:

From the WHO Fact Sheet: http://www.who.int/mediacentre/factsheets/fs117/en/index.html
From the WHO http://www.who.int/denguecontrol/en/index.html
Jorge R. Rey from the University of Florida Institute of Food and Agricultural Science has written fantastic pieces that have served as primary background sources and are freely available at: http://edis.ifas.ufl.edu/in699 (What is Dengue?).
Dengue Vaccine Initiative: http://www.denguevaccines.org/