

Detecting Dengue in the Lab and Field

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 four 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. With additional funding from the National Institute 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?

Next Generation Sunshine State Standards - Science

Standard	Lesson							
	1	2	3	4	5	6	7	8
SC.912.L.14.4: Compare and contrast structure and function of various types of microscopes.	X							
SC.912.L.14.6: Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.		X					X	
SC.912.L.14.52: Explain the basic functions of the human immune system, including specific/ nonspecific immune response, vaccines, and antibiotics.			X	X		X	X	
SC.912.L.16.10: Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.			X	X	X		X	
SC.912.L.16.11: Discuss the technologies associated with forensic medicine and DNA identification, including RFLP analysis.					X	X	X	
SC.912.L.16.12: Describe how basic DNA technology (gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).					X	X	X	
SC.912.L.17.4: Describe changes in ecosystems resulting from seasonal variations, climate change and succession.	X						X	
SC.912.L.17.15: Discuss the effects of technology on environmental quality.	X						X	
SC.912.L.18.1: Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.			X	X	X	X	X	
SC.912.N.1.1: Define a problem based on a specific body of knowledge, for example: biology, and do the following: <ol style="list-style-type: none"> 1. pose questions about the natural world, 2. conduct systematic observations, 3. examine books and other sources of information to see what is already known, 4. review what is known in light of empirical evidence, 5. plan investigations, 6. use tools to gather, analyze, and interpret data, 7. pose answers, explanations, or descriptions of events, 8. generate explanations that explicate or describe natural phenomena (inferences), 9. use appropriate evidence and reasoning to justify these explanations to others, 10. communicate results of scientific investigations, and evaluate the merits of the explanations produced by others. 	X						X	
SC.912.N.1.2: Describe and explain what characterizes science and its methods.	X						X	
SC.912.N.1.6: Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.	X						X	
SC.912.N.3.5: Describe the function of models in science, and identify the wide range of models used in science.			X			X		
SC.912.N.4.1: Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.						X	X	
SC.912.N.4.2: 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.							X	

Vocabulary

Adult- The fully developed mature form.

Egg- The first stage in the mosquito life cycle.

Larva- The immature, wingless form that hatches from an egg. Purpose is to eat and grow.

Pupa- The non-feeding stage in the life cycle during which the larva changes to the adult form.

Raft- Cluster of eggs laid on the surface of permanent water.

Siphon- Tube used by the larva to breathe air.

Trumpet- Tube used by the pupa to breathe air.

Tumbler- Common name for the mosquito pupa.

Wiggler- Common name for the mosquito larva.

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 several 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 (DEN) comprises four distinct, but closely related, serotypes (DEN-1, DEN-2, DEN-3 and DEN-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 DEN-2 and DEN-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>

Dengue guidelines, for diagnosis, treatment, prevention and control. This manual provides health professionals and programmes managers with the latest recommendations. Ref: ISBN 978 92 4 1547871
http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf

IFAS: The Mosquito and What is Dengue? Jorge R. Rey

Dengue Vaccine Initiative: <http://www.denguevaccines.org/>

Lesson Sequencing Guide

Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. Below is a suggested pacing guide that can be used when planning to use this curriculum.

45 minute periods

Need to change. Stations is now own lesson – lesson 2.

	Day 1	Day 2	Day 3	Day 4	Day 5
Week 1	Lesson 1 ^A Introduction (15 minutes)	Lesson 1 Breeders and Stations (45 minutes)	Lesson 1 Observations (5 minutes) Lesson 2 (20 minutes) Lesson 3 (15 minutes)	Lesson 1 Observations (5 minutes) Lesson 4 (45 minutes)	Lesson 1 Observations (5 minutes) Lesson 5 (45 minutes)
Week 2	Lesson 1 Observations (5 minutes) Lesson 6 ^B (30 minutes)	Lesson 1 Observations (5 minutes) Lesson 7 (45 minutes)	Lesson 1 Observations (5 minutes) Lesson 7 ^B (15 minutes)	Lesson 1 Observations (5 minutes) Lesson 8 ^B (30 minutes)	Lesson 1 Observations (5 minutes)
Week 3	Lesson 1 Observations (5 minutes)	Lesson 1 Observations (5 minutes)	Lesson 1 Observations (5 minutes)	Lesson 1 Observations (5 minutes)	Lesson 1 Identification (30 minutes)
Week 4					

^A - Lesson 1 is an ongoing activity as students observe the life cycle of the mosquito. This lesson could be completed separately from the unit or omitted, as needed.

^B - Lesson 6, Lesson 7 (day two) and 8 can be given as homework.

If Lesson 7 is completed before Lesson 1, students can then consider if they have carriers in their community, and what steps could be taken to minimize the risk of an outbreak.

PDB: <http://www.rcsb.org/pdb/101/motm.do?momID=103> molecule of the month

Module 1: Oh No! No More Mosquito!

Lesson 1: What's Buzzing in Your Backyard?

KEY QUESTION(S): What is the lifecycle of the mosquito? Where are mosquitoes found?

OVERALL TIME ESTIMATE:

30 minutes for initial set-up;

5 minute daily observations for ~2 weeks;

30 minute wrap-up

*Since mosquitoes are most common during warm, humid times of the year, if having students collect samples, plan this activity for the beginning or end of the school year in the Southeastern US.

LEARNING STYLES: Visual, kinesthetic, and auditory

VOCABULARY:

Egg

Larva

Pupae

Adult (Imago)

Arthropod

Vector

LESSON SUMMARY: This guided inquiry lesson allows students to discover where mosquitoes live and breed. They are given minimal initial instruction on how to collect water samples (giving them a big clue that water plays an important role in mosquito development) and guidance using mosquito breeders, but formulate questions and discover answers on their own. This unit specifically focuses on Dengue virus and its mosquito vectors: *Aedes aegypti* and *Aedes albopictus*. Students will discover the basic life cycle of the mosquito and by doing so, formulate ideas about how disease spreads and how to interrupt its spread.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

Identify mosquito developmental stages

Locate potential breeding sites for mosquitoes

Estimate the length of time needed for a mosquito to reach maturity

Estimate survival rate to next developmental stage

Use a stereoscope

Identify mosquito species common in their community

Relate the mosquito life cycle to halting disease spread

STANDARDS:

MATERIALS:

Source	Student workstation	Number required per group	Number required for class
BioQuip http://www.bioquip.com/ Cat no 1425DG (16oz) \$9.95 ea	Mini mosquito breeders	1	variable
Fisher Scientific	Forceps Slide mounting gel (or clear nail polish)		
	Disposable pipets		
	Small petri dishes		
Carolina Biological	<i>Culex</i> eggs		
	Larval food source (from Carolina or dry milk, ground dog food)		
	Deep well slides and cover slips		
	Stereoscopes		
	Mosquito life cycle slides		
UF IFAS Bookstore	Common mosquitoes of Florida ID cards	1	
	Mosquito life cycle chart		1
Grocery store	Spring water		1 gallon
Classroom laboratory	Thermometers	1	
Classroom laboratory	Compound microscopes	(optional)	
Classroom laboratory	Sharpie	1	
Classroom laboratory	Scissors	1	
Classroom laboratory	Colored pencils/crayons	Assortment	

BACKGROUND INFORMATION:

Mosquitoes are common arthropod vectors of disease including Dengue, West Nile, and Malaria, however, only certain species of mosquitoes are vectors of disease. Most of the species found in our backyards are beneficial insects, serving as pollinators and also as a food source for fish and other insects. This lesson specifically focuses on Dengue virus and its mosquito vectors: *Aedes aegypti* and *Aedes albopictus*. Students will discover the basic life cycle of the mosquito and by doing so, formulate ideas about how disease spreads and how to interrupt its spread.

ADVANCE PREPARATION: The samples students bring in may not be ideal, so it is recommended that the instructor also collect field samples. As a control, you may wish to order *Culex* eggs or larvae from a supply company so students can chart the life cycle more precisely, starting with a known number of

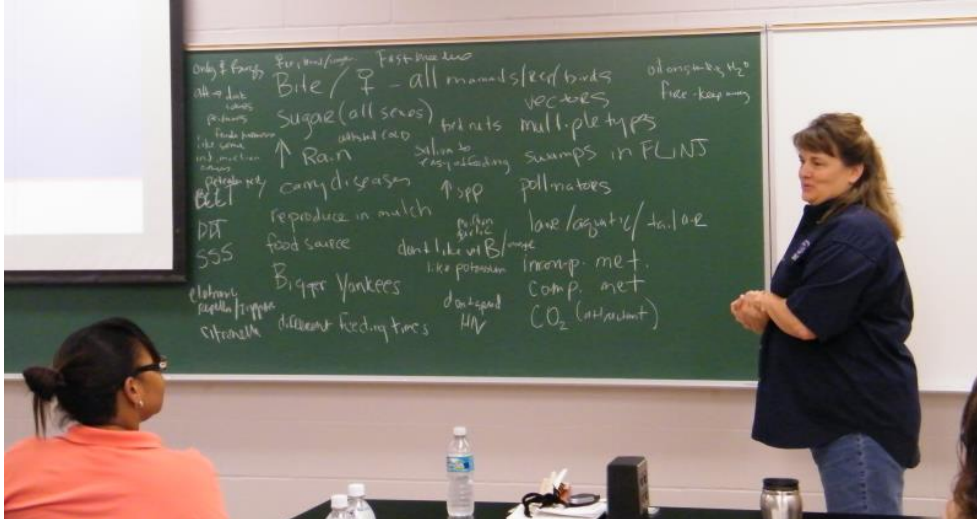
larvae and determining the number that reach maturity. It also allows an excellent opportunity to model scientific procedures and inquiry.

**Implementation tip: Engage your students by having larva on your desk or at the front of the room several days before the starting the unit. Allow them to observe and talk amongst themselves about the wiggling things.*

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Day 1- Introduction (15 minutes) (Consider introducing the project and allowing several days –perhaps over a weekend- for students to collect their samples.)

1. Tell the students they will be investigating the life cycle of mosquitoes, and determining which species of mosquitoes are in their community.
2. Activate prior knowledge by asking the students what they know or think they know about mosquitoes. Record this information on a chalkboard or flip chart to refer to later in the unit. (At this point, misconceptions don't need to be clarified or corrected, although the instructor should make a note to self to address the misconception during the unit.)



3. Lead the students to the correct idea that mosquito larvae is found in water and that it is often referred to as “wriggling water”.
4. Ask each student (or student pair) to bring in a water sample (~250ml = ~1 cup) that they believe might contain mosquito larvae.

Day 2 – Setting up mosquito breeders (15 minutes)

1. Determine if students are going to work in pairs or in groups (largely dictated by the number of mosquito breeders available. If number of breeders is an issue, larva can be left in a plastic container, with a lid or mesh, for observations. The mosquito breeder allows for capture and id of adult mosquitoes. Once adults have fallen in water, they are more difficult to recover and id, although not impossible.)
2. Ensure each group has at least one water sample.
3. Distribute the *Mosquito Log Cover Sheet* to each group and instruct them to complete it.

Day 3-14 – Daily observations (5 minutes)

As students arrive each day, they need to observe their mosquito culture and record their observations on the *Daily Mosquito Log Sheet*. Keep a stack of these available for students. If participation is an issue, consider having the students take turns being the lead caretaker. As students inquire about conditions (do they need to eat, do we need to change the water, should they be in the sun) allow them to come up with an answer and experiment. In a common station, have the following accessible:

- *Daily Mosquito Log Sheets*
- Food (dry milk, yeast, ground dog food, tiny bit of dog biscuit, commercial mosquito food – a tiny pinch every 2-3 days to prevent film from covering the surface)
- Spring water
- Thermometers
- Forceps
- Hand lens
- Stereoscope
- Deep-well slides
- Petri dishes

Day 15 – Adult identification and wrap-up (45 minutes)

*Safety note! Take care not to release live mosquitoes. Vertical transfer does occur. (Female mosquitoes can pass virus to their eggs, therefore newly emerged females can be infectious.)

1. Once adults have entered the top section, they will live for one to four days. Once there are no adults moving, the top can be removed and the adults observed under the stereoscope. (To expedite the identification process, adult mosquitoes can be killed by placing the top section in the freezer.)
2. Students will transfer their adults using forceps to a deep well slide or regular slide. If using a regular slide, they can affix their mosquito using clear nail polish or other slide mounting gel.
3. Students should identify the genus and gender of the mosquito using the id cards.
*Simplified version- Ask students to determine if it is a dengue mosquito - *Ae. aegypti* / *Ae. albopictus* - or not.
4. Encourage all members to id each mosquito and record their individual answers. Majority wins.
5. Students may become frustrated trying to identify the mosquitoes. Remind them to do their best and help each other out.

ASSESSMENT SUGGESTIONS:

Daily Mosquito Log Sheets should be checked for completion and to ensure correct procedure. Students may be given participation points as well.

Writing prompt: Use the information you have gained related to mosquitoes and disease and develop a safe, sustainable and environmentally-sound plan for the school to minimize the spread of arthropod-borne disease at your school.

EXTENSIONS:

ACTIVITIES: Have students compile class data and create a graphical representation of the type and number of mosquito genus identified.

LITERATURE:

RESOURCES/REFERENCES:

http://www.osceola.org/mosquitocontrol/129-6426-0/mosquito_life_cycle.cfm

http://commons.wikimedia.org/wiki/File:Culex_mosquito_life_cycle_nol_text.svg

Mosquito Log Cover Sheet (day one)

Group member names: _____

Date mosquito sample collected: _____

Location and description of collection site: _____

Date transferred to mosquito breeder: _____

Water temperature: _____ Air temperature: _____

Location and description of placement in classroom: _____

Life stage of mosquitoes: _____ Approximate # of organisms: _____

Additional observations: _____

Mosquito Log Cover Sheet (day one)

Group member names: _____

Date mosquito sample collected: _____

Location and description of collection site: _____

Date transferred to mosquito breeder: _____

Water temperature: _____ Air temperature: _____

Location and description of placement in classroom: _____

Life stage of mosquitoes: _____ Approximate # of organisms: _____

Additional observations: _____

Daily Mosquito Log Sheet

Caretaker name: _____

Date: _____

Air temp: _____

Life stage of mosquitoes: _____

Approximate # of organisms: _____

Life stage of mosquitoes: _____

Approximate # of organisms: _____

Life stage of mosquitoes: _____

Approximate # of organisms: _____

Additional observations: _____

Changes to care and keeping of mosquitoes: _____

Daily Mosquito Log Sheet

Caretaker name: _____

Date: _____

Air temp: _____

Life stage of mosquitoes: _____

Approximate # of organisms: _____

Life stage of mosquitoes: _____

Approximate # of organisms: _____

Life stage of mosquitoes: _____

Approximate # of organisms: _____

Additional observations: _____

Changes to care and keeping of mosquitoes: _____

LESSON TWO: Neato Mosquito Learning Stations

KEY QUESTION(S): What are the stages of the mosquito life cycle?

OVERALL TIME ESTIMATE:

30 minutes set-up

45 minutes classroom

LEARNING STYLES: Visual, auditory, and kinesthetic

VOCABULARY:

Egg

Larva

Pupae

Imago

Arthropod

Vector

LESSON SUMMARY: Students explore the stages of the mosquito life cycle as they move through multiple learning centers. Prompted by station cards and a scavenger-hunt style worksheet, students engage in multiple learning experiences by viewing microscope slides, videos, and illustrative information posters. Students will also practice laboratory techniques and use of laboratory equipment including disposable pipettes, stereoscopes, and microscopes.

STUDENT LEARNING OBJECTIVES WITH STANDARDS:

The student will be able to...

Identify the three body parts common to all insects

Identify mosquito developmental stages

Differentiate male and female adult mosquitoes based on anatomical structures

Describe how mosquito mouthparts are adapted for feeding on liquids.

Describe the foods used by adult male and female mosquitoes.

Describe how female mosquitoes locate hosts.

Describe the mechanics of blood feeding.

Explain why mosquito bites itch.

Use a stereoscope

Explain why female mosquitoes need to take a blood meal

Utilize written and internet sources

Identify mosquito genus common in their community

Relate the mosquito life cycle to halting disease spread

MATERIALS:

Station	Description	Materials		
A	<i>Culex</i> egg raft under a stereoscope	Culex egg raft from Carolina 144470 Mosquito (Culex) Egg Raft,		

		<p>Living \$15.75 Stereoscope Deep well slide 603730E Slides, Carolina™ Deep-Well, Set of 5, \$13.95 or small petri dish</p>		
B	<p><i>Culex</i> larvae under a stereoscope (if possible, provide disposable pipettes and allow students to place the larvae on a slide themselves for viewing)</p> <p>*Note: Newly hatched larvae are very tiny and difficult to see. Samples from home or larvae that are several days old should be used for this station.</p>	<p>Culex larvae 144476 Mosquito (<i>Culex</i>), Living, Larvae, Lab Reared, Pack of 25, \$17.50 (or start one egg raft approximately one week before the lesson) Stereoscope Disposable pipettes Deep well slides 603730E Slides, Carolina™ Deep-Well, Set of 5, \$13.95 or small petri dishes</p>		
C	<p>Adult mosquito under a stereoscope / hand lens</p>	<p>Adult mosquitoes Male/female: 308094 Culex Male and Female, w.m. Microscope Slide, \$12.75 (can use <i>Culex</i> reared in the classroom or adults from self collections. Can be kept in a small container. Allow students to carefully place them on the slide or have it already set up with different mosquitoes so they can compare different species) Stereoscope Forceps</p>		
D	<p>Prepared slides of mosquito life cycle (from Carolina Biological)</p>	<p>308112 Mosquito Life Cycle, w.m. Microscope Slide, \$14.40</p>		
E	<p>Common mosquito of Florida id cards. Find five that are found in your city/county.</p>	<p>http://ifasbooks.ifas.ufl.edu/p-273-common-mosquitoes-of-florida-id-deck.aspx</p> <p>Common Mosquitoes of Florida ID Deck, SP370, \$12</p>		

F	<p>Segment of Dirty Jobs: Mosquito Control Officer (minutes 27:35-43:35) (Can we break this into two parts? One outside control measures; second in the lab?)</p>	<p>http://www.amazon.com/gp/product/B001PO118K Available for purchase through Amazon and iTunes; streaming with Netflix Season 2, Episode 29, Aired 12/19/06</p> <p>http://videos.howstuffworks.com/discovery/426-dirty-jobs-mosquito-control-video.htm hatching eggs – 2 minutes – can be embedded</p> <p>http://videos.howstuffworks.com/discovery/28298-dirty-job-mosquito-control-video.htm fish and larvacide in an abandoned pool</p>		
	<p>Click and learn HHMI BioInteractive: Stopping Mosquito-Borne Disease</p>	<p>http://www.hhmi.org/biointeractive/disease/Dengue/01.html (first section 1-10 life cycle; 11-14 how to stop dengue; 15-36 eradication program in Brazil)</p>		
G	<p>PPT slide from Dr. Connolly with the way virus moves around during blood meal</p>			
	<p>Mosquito life cycle cut out (http://www.mosquitoes.org/downloads/cut-out%20life%20cycle.pdf)</p>			
	<p>Crossword, word search, word scramble (http://www.mosquitoes.org/educ_materials.htm)</p>			
	<p>Scavenger hunt to complete the worksheet, use posters in the classroom as well (http://www.mosquitoes.org/downloads/MosqFacts.pdf and life cycle above)</p>			
	<p>Video from iStockphoto. What stages are represented? Draw them.</p> <p>Discover magazine: mosquito life cycle http://videos.howstuffworks.com/discovery/2</p>			

	8333-discover-magazine-mosquito-life-cycle-video.htm			

BACKGROUND INFORMATION:

The Mosquito¹

Jorge R. Rey²

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2. Jorge R. Rey, professor, Entomology and Nematology Department, Florida Medical Entomology Laboratory, Vero Beach, Cooperative Extension Service, Institute of Food and Agricultural Sciences. University of Florida, Gainesville, FL 32611

What is a Mosquito?

Mosquitoes belong to the insect order Diptera, family Culicidae. Approximately 167 species of mosquitoes belonging to 13 genera are found in the United States; of these, 80 species occur in Florida. It is estimated that there are more than 2500 species of mosquitoes worldwide. Mosquitoes can be identified by noting that as all true flies they only have one pair of functional wings (most insects have two), and that they have a long, piercing proboscis, and scales on the veins of their wings.

Mosquitoes have a complex life cycle which includes the egg stage, several larval stages (or instars), a pupal stage, and an adult stage. All of the immature stages are aquatic, and adult females return to water to lay their eggs. The name mosquito comes from the Spanish "musketas," meaning "little fly". The native Hispanic-Americans called them "zancudos", a term that is still used in parts of South and Central America; it means "long-legged".

Mosquito Life Cycle

Eggs - Depending upon the particular species, the female mosquito lays her eggs either individually or in groups called **rafts**. Any water-holding area, such as tree holes, ponds, puddles, ditches and artificial containers such as discarded tires and flower pots can serve as a mosquito breeding site.

Some mosquitoes (floodwater mosquitoes) lay their eggs in moist substrates without standing water. These eggs are usually resistant to desiccation (drying) and hatch when flooded by rainfall, tides, or water diversion to the area. A Florida example would be *Culex nigripalpus*. Other species (pool breeders, standing water mosquitoes) lay their eggs only upon standing water and the eggs are not usually resistant to drying. The eggs are placed directly upon the surface of the water or along the edges of

pools or reservoirs. In either case, water must remain on the surface long enough for the mosquitoes to hatch and complete development (see below).

Larvae - After hatching from the egg, the mosquito **larva** undergoes a series of growth stages with continuous feeding that eventually will transform the insect from a swimming aquatic form to a flying terrestrial one. Because the larvae are covered in a hard protective skin called the cuticle, they must undergo a series of molts in order to grow. When ready to molt, the larvae then shed the old exoskeleton and the new one hardens when exposed, to protect the larvae's internal organs. Mosquito larvae, also known as "**wrigglers**" undergo four such molts. The stages between molts are called instars and are numbered from I to IV.

Pupae - After the fourth instar, the development of the future mosquito adult is about to start. The process involves the breakdown of the larval organs and their replacement with the adult ones. During this process, the mosquito takes a new shape-- the **pupa**. The pupa can be considered to be a sealed envelope, where the adult organs are developed from larval tissues. The pupa does not feed or eliminate waste products. Its only contact with the outside is through breathing tubes (**trumpet**) located on the thorax. After 3 or 4 days, the adult mosquito emerges from the pupa, and after a period of rest, unfolds its wings and flies away.

Adults - The male mosquito will usually emerge first, and will linger near the breeding site, waiting for the females. On average, a female mosquito will live 3-6 weeks, but can live up to 5 months. The male's life span is much shorter. Both adult male and female will feed on nectar and plant fluids, but it is only the female that will seek a blood meal, which most species need in order to develop their eggs.

Female mosquitoes lay multiple batches of eggs and most species require a blood meal for every batch they lay. Females of some species can develop a limited number of egg batches (usually 1) without taking a blood meal, a quality known as "autogeny". In tropical regions, adult mosquitoes are active throughout the year, but in other areas they become inactive when the temperature drops below 60°F and usually enter hibernation when the seasonal cool temperatures arrive. A few mosquito species hibernate as larvae, usually buried in moist swamp muds, but most overwinter either as eggs laid by the last generation, or as adult, mated females that spend the winter in protected locations such as hollow trees, animal burrows, attics, etc.

Mosquito Bites

Mosquitoes suck blood to obtain proteins and other nutrients necessary for egg development, so only female mosquitoes "bite". Actually, mosquitoes do not really bite; "sting" is probably a better term. Once it locates a host, the female will probe the skin for a blood capillary then insert its very thin and sharp proboscis through the skin into the blood vessel and begin sucking blood. In the process, the mosquito will inject a small amount of saliva, which functions both as a lubricant for proboscis insertion and as an anticoagulant (prevents blood clotting). It is the proteins in the saliva that evoke an immune response and cause the swelling and itching.

When a mosquito bites someone, it does not inject its own blood or the blood of an animal or person it has bitten before into the next person it bites. Salivary fluid injection and blood uptake occur through

separate passageways. Diseases are transmitted only if the disease organism reproduces in the mosquito, or at least survives long enough to infect the salivary glands.

Avoiding Mosquito Bites

Mosquito bites can be avoided in several ways:

- Around the home: Remove all water holding containers that may serve a mosquito breeding sites. If containers can't be removed, drain them and cover them so that they don't collect water, or flush every 2 or 3 days. Use natural predators such as mosquitofish in ornamental fountains or ponds, or use an approved mosquitoicide. (see <http://eis.ifas.ufl.edu/prevent.htm> for more information).
- Avoid outdoor activities when mosquitoes are most active. Specific times vary with the mosquito species, but the hours around dawn and dusk are particularly important.
- Wear protective clothing (long sleeves, socks and long pants).
- Use repellents that contain DEET (see <http://www.acponline.org/journals/annals/01jun98/mosquito.htm>).

ADVANCE PREPARATION:

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

ASSESSMENT SUGGESTIONS:

EXTENSIONS:

ACTIVITIES:

LITERATURE:

RESOURCES/REFERENCES:

<http://www.cdc.gov/ncidod/dvbid/arbor/neato.htm>

Module Two – On the Case: Investigating an Outbreak

Lesson 3: What Ails You? The Investigation Begins

KEY QUESTION(S): What are the steps to diagnosing dengue?

OVERALL TIME ESTIMATE: 30 minutes

LEARNING STYLES: Visual and auditory

VOCABULARY:

Dengue fever

Epidemiology

Case report

Endemic

LESSON SUMMARY: 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. After a return trip and visit to the emergency room, initial testing for dengue will be conducted by the students in the next lesson.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

Interpret a case report

Understand that investigating a disease cause is a detailed process

Identify some of the intricacies in investigating a disease cause

Recognize it can be difficult to diagnosis an illness

STANDARDS:

MATERIALS:

Copies of the case report per student

Copies of the epidemiological log per student

BACKGROUND INFORMATION:

Endemic dengue is rare in the United States. There have been cases reported along the Texas/Mexico border as well as an outbreak in Hawaii, and spotted reports usually as a result of travel to other countries. The outbreak in Key West was alarming as it served as a warning signal as to what was possible. Key West is rather removed from the rest of the Florida peninsula and fortunately, *Aedes* mosquitoes aren't known to travel great distances. However, it is a distinct possibility that an infected mosquito or person could carry the virus into the rest of the state and allow dengue to establish itself in the mosquito population. Once established, it would be very difficult to eradicate, particularly in a state with such a high amount of tourist and global travel. The tropical disease dengue is often misdiagnosed since few cases are seen in the United States. Additionally, its early flu-like symptoms are often dismissed by the patient.

ADVANCE PREPARATION:

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

30 minutes

1. Distribute copies of the Case Report per student.
2. Tell the students this is the actual case report as recorded by the CDC. Ask students to read the case report silently.
3. Tell the students they are now an epidemiologist on the case. They need to review her history and record her symptoms, tests ordered, and results as they are available. They will continue to fill in the epidemiological report as they move through the unit and more information is learned.
4. (5-10 minutes) Allow the students to work in pairs to complete as much of the chart as they can.
5. Circulate to check for understanding and remind them where the dictionaries are located should they need to look up a word.
6. When all student pairs have finished, go through the epidemiological report together, calling on student pairs to give answers.

ASSESSMENT SUGGESTIONS:

- Participation grade may be given.
- Students will complete the first part of the Epidemiology Report, and these can be graded at the conclusion of the unit.

EXTENSIONS:

ACTIVITIES:

LITERATURE:

RESOURCES/REFERENCES:

Lesson 3: The Investigation Begins – Student Worksheet

In this activity, you will take on the role of an epidemiologist. Review the patient's history and record her symptoms, tests ordered, and results as they are known. You will continue to fill in the epidemiological report as you move through the unit.

Case 1.

*On **August 11, 2009**, a previously healthy woman aged 34 years from Rochester, New York, went to her primary-care provider after 1 day of fever, headache, malaise, and chills. A urine analysis revealed bacteruria (bacteria in urine) and hematuria (red blood cells in urine), and she was treated for a presumptive urinary tract infection.*

*Two days later, on **August 13**, she returned to her primary-care provider with a worsening headache, retro-orbital pain exacerbated by eye movement, and complaints of feeling light-headed, although her fever had resolved. Physical examination determined that she was alert and oriented but had substantial discomfort from her headache; further neurologic evaluation determined that the patient had loss of balance when asked to close her eyes. She was referred to a local emergency department for further evaluation and management.*

At the emergency department, she had normal vitals of a temperature of 98.8°F (37.1°C), heart rate of 85 beats per minute, blood pressure of 117/96 mmHg, and respiratory rate of 16 breaths per minute. A complete blood cell (CBC) count revealed a low white blood cell count of 3,900/μL (normal: 4,500--10,500/μL), a normal hematocrit of 43%, and a low platelet count of 115,000/μL (normal: >150,000/μL). Her evaluation included an unremarkable computed tomography (CT) scan of the head and a lumbar puncture. The patient's light-headedness resolved, and she was discharged after a 7.5-hour stay in the emergency department.

*On **August 17**, the woman returned to her primary-care provider, saying, "I don't feel right." On examination she had a temperature of 98.8°F (37.1°C), heart rate of 76 beats per minute, blood pressure of 122/60 mmHg, trace pedal edema (swelling) bilaterally, and petechiae (small (1-2mm) red or purple spots on the body) on her lower extremities.*

EPIDEMIOLOGICAL REPORT

Patient Case #: _____ Gender: Male Female Age: _____

Home address: _____ Recent travel: _____

Date	Symptoms	Sample source	Test performed	Result	Diagnosis?
	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:				
	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:				
	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:				
	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:				

Draw or affix image of ELISA below.

Draw or affix image of PCR/ gel electrophoresis below.



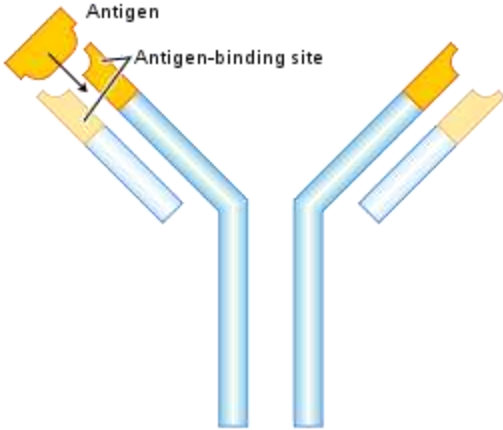
EPIDEMIOLOGICAL REPORT: Answer Key

Patient Case #: 1 Gender: Male Female Age: 34

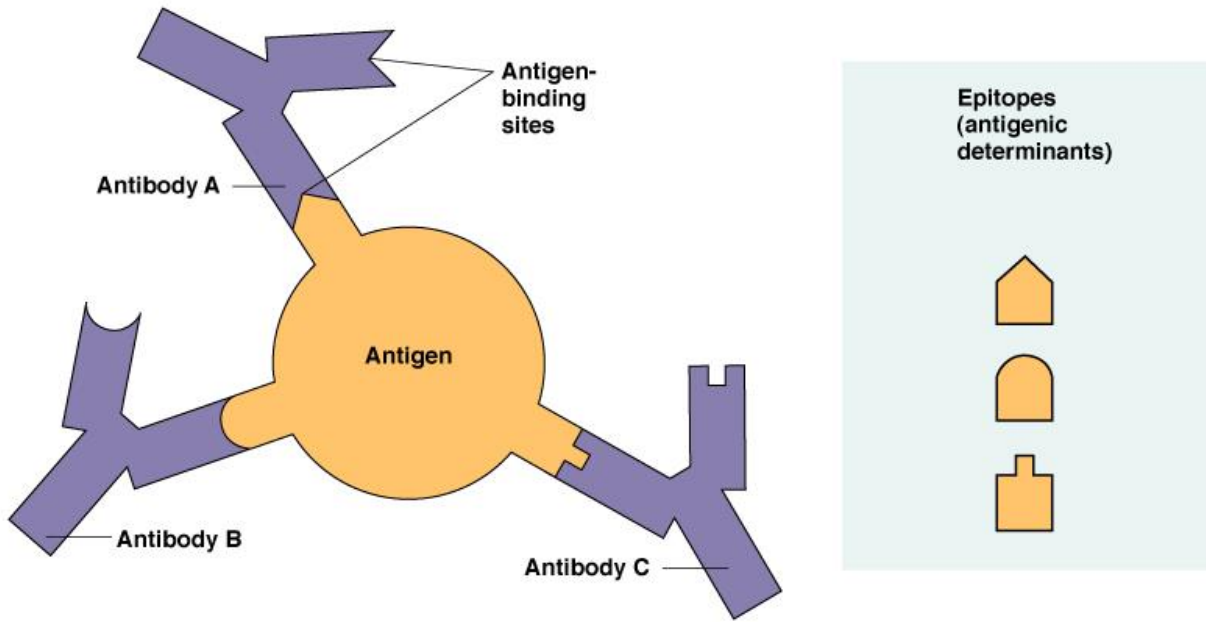
Home address: Rochester, New York Recent travel: One week in Key West

Date	Symptoms	Sample source	Test performed	Result	Diagnosis?
August 11	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:	Urine	Urine analysis	Bacteruria, hematuria	Urinary tract infection
August 13	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:	Lumbar puncture = cerebral spinal fluid	RT-PCR	Positive for DENV-1	On August 13 – unknown Later testing revealed Dengue fever serotype 1
August 17	<input type="checkbox"/> Chills <input type="checkbox"/> Malaise <input type="checkbox"/> Fever <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Headache <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Rash <input type="checkbox"/> Light-headed <input type="checkbox"/> Swelling Other:	Serum (blood)	MAC ELISA	Positive for dengue IgM antibodies	On August 17- Dengue suspected. Testing confirmed diagnosis.
September 3	Recovered	Serum (blood)	MAC ELISA	Positive for dengue IgM antibodies	Recovered. Circulating dengue antibodies.

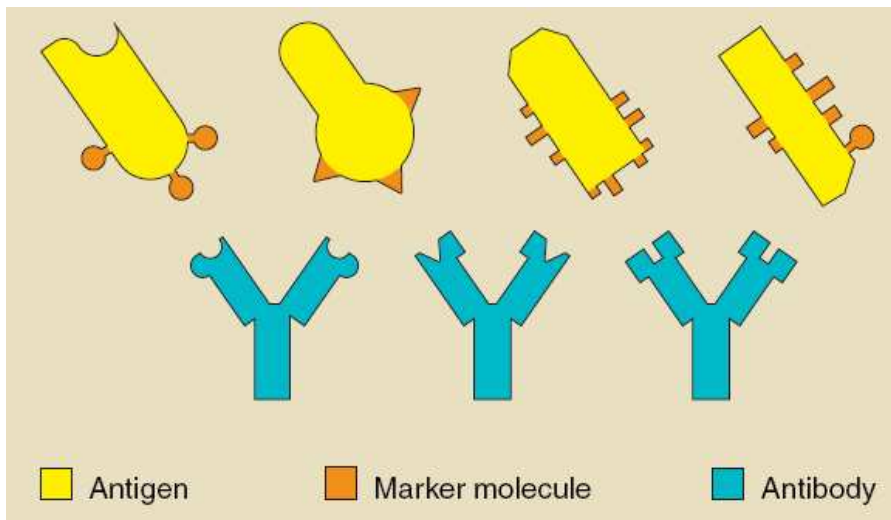
Antigens



Antibody



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LESSON 4: Steps of an ELISA

KEY QUESTION(S): What is an ELISA? How is it used as a diagnostic test?

OVERALL TIME ESTIMATE: 15 minutes

LEARNING STYLES: Visual, kinesthetic, and auditory

VOCABULARY:

ELISA

Antigen

Antibody

Substrate

Primary antibody

Secondary antibody

LESSON SUMMARY: 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.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

Sequence steps of an ELISA test

Define ELISA

Explain the use of an ELISA to aid in disease diagnosis

Describe antigen/antibody interaction

Diagram antigen/antibody interaction

STANDARDS:

MATERIALS:

Steps of an ELISA cards, cut (laminated for repeated use)

Steps of an ELISA student worksheet, per student pair (laminated for repeated use)

BACKGROUND INFORMATION:

The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be

covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

ADVANCE PREPARATION:

Instructor may wish to fashion this activity as a file folder game.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Review or introduce the following key points about antibodies and antigens:
 - Antigens are foreign proteins which cause the immune system to generate antibodies.
 - Specific antibodies are produced for each antigen. They bind like a lock and key.
 - There are different antibodies produced by the human immune system (IgG, IgM, IgE, etc) but all have the same basic starting structure: a Y. At the top of the Y is the part that recognizes a specific antigen.
 - The part of the antigen that binds with the antibody is referred to as the epitope. In this picture, it is the orange parts.
 - Show the students that the tops of the antibody “Y” fits with certain epitopes on the antigens. Some antigens have multiple epitopes, so they are recognized by different antibodies (kind of like a back-up system).
 - Tell students scientists have developed diagnostic assays that utilize the unique and specific binding properties of antibodies and antigens.
 - Introduce the idea that antibodies can serve as antigens as well and in diagnostic assays we create antibodies that recognize other antibodies as antigens or a protein which it is specific for.
 - Primary antibodies recognize the original antigen we are testing against. Secondary antibodies recognize the first (primary) antibody. Using both increases sensitivity.
2. Arrange students in pairs.
3. Distribute Steps of an ELISA cards and student worksheets to each pair.
4. Tell them to follow the directions on the worksheet.
5. (5-10 minutes) Allow student pairs to complete activity.
6. Review the steps together, clarifying as needed.
7. Show the video to reinforce how an ELISA is performed.
<http://www.youtube.com/watch?v=RRbuz3VQ100&feature=related>

ASSESSMENT SUGGESTIONS:

Instructor can visually observe correct completion of the activity.

EXTENSIONS:

ACTIVITIES:

Antibody/antigen matching game to illustrate specificity of bonding through epitopes.

LITERATURE:

RESOURCES/REFERENCES:

ELISA video: <http://www.youtube.com/watch?v=RRbuz3VQ100&feature=related>

Lesson 3: Steps of an ELISA- Student Worksheet

A common test used to detect if a patient has been exposed to a virus such as HIV, Dengue, or West Nile 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. ELISA tests usually take place in plastic plates containing wells, or depressions.

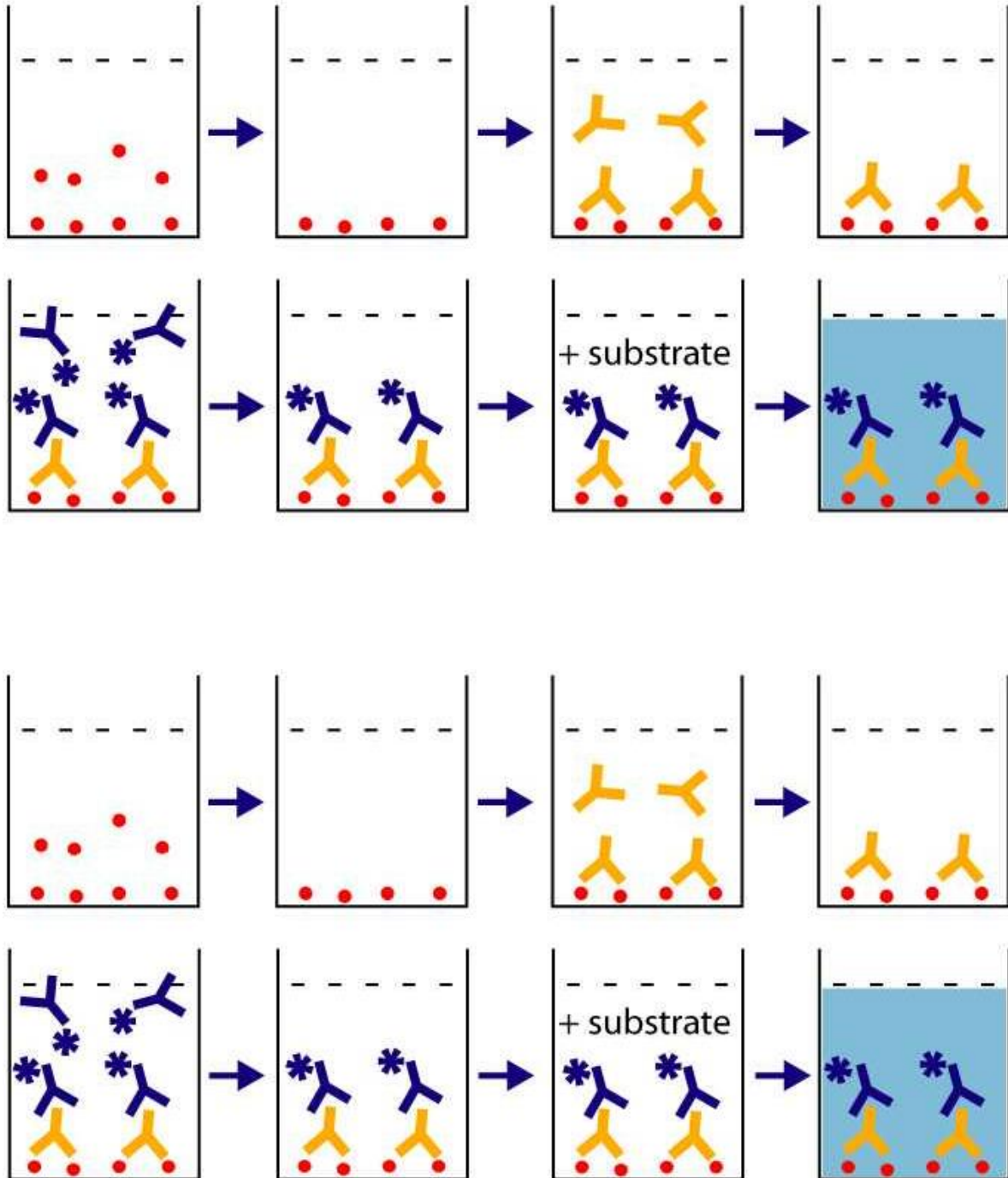
Match the statements and images below to sequence the steps of an ELISA test.

	Virus proteins (antigens) are added to wells of a 96-well plate.
	The antigens bind to the plastic, coating the bottom of the wells.
	The primary antibody is added to the well. In the case of the dengue ELISA, the primary antibodies (IgM) are from the patient's serum sample.
	Excess antibody is washed away, leaving only antibodies bound to the antigens behind. This wash removes excess antibodies that are unbound and prevents non-specific binding.

	<p>A secondary antibody is added to the wells. This antibody recognizes the patient IgM antibodies, bound to the antigens. The secondary antibody also has a colorimetric tag attached.</p>
	<p>Excess secondary antibody is washed away, leaving only secondary antibodies, bound to the patient IgM antibodies. This wash removes excess antibodies that are unbound.</p>
	<p>A substrate is added to the wells.</p>
	<p>Bound secondary antibody containing a colorimetric tag will cause a color change when exposed to the substrate. A color change indicates a positive reaction.</p>

Lesson 3: Steps of an ELISA- cards

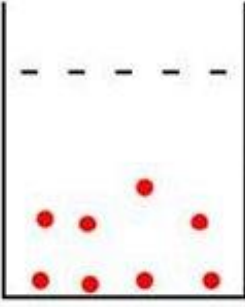
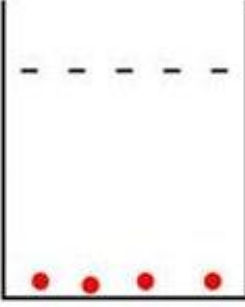
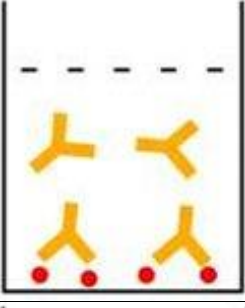
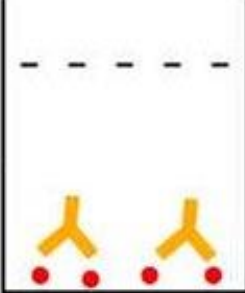
Make one set of eight cards for each group. Cut on both sides of arrows and between rows to separate into eight cards, each representing a step in the ELISA reaction.



Lesson 3: Steps of an ELISA- Teacher Answer Key

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. ELISA tests usually take place in plastic plates containing wells, or depressions.

Match the statements and images below to sequence the steps of an ELISA test.

	Virus proteins (antigens) are added to wells of a 96-well plate.
	The antigens bind to the plastic, coating the bottom of the wells.
	The primary antibody is added to the well. In the case of the dengue ELISA, the primary antibodies (IgM) are from the patient's serum sample.
	Excess antibody is washed away, leaving only antibodies bound to the antigens behind. This wash removes excess antibodies that are unbound and prevents non-specific binding.

	<p>A secondary antibody is added to the wells. This antibody recognizes the patient IgM antibodies, bound to the antigens. The secondary antibody also has a colorimetric tag attached.</p>
	<p>Excess secondary antibody is washed away, leaving only secondary antibodies, bound to the patient IgM antibodies. This wash removes excess antibodies that are unbound.</p>
	<p>A substrate is added to the wells.</p>
	<p>Bound secondary antibody containing a colorimetric tag will cause a color change. A color change indicates a positive reaction.</p>

LESSON 4: Testing for Dengue Antibodies

KEY QUESTION(S): Is case number 1 positive for dengue virus?

OVERALL TIME ESTIMATE: 45 minutes

LEARNING STYLES: Visual and kinesthetic

VOCABULARY:

ELISA

Antigen

Antibody

Substrate

Primary antibody

Secondary antibody

LESSON SUMMARY: 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.

STUDENT LEARNING OBJECTIVES:

Perform an ELISA test

Understand (Explain) the use of biotechnology to diagnose disease

Know (Recognize) that an ELISA is an antibody-based test rather than nucleic acid

Explain the steps of an ELISA

Consider (Propose) other uses of an ELISA

STANDARDS:

MATERIALS:

ELISA test (BioRad's Biotechnology Explorer ELISA Immunoexplorer Kit Catalog # 166-2400EDU Protocol III – Antibody test)

OR

Fluorescent ink pen

12-well microplate strips

Assorted 1.5 or 2.0ml microfuge tubes

Microfuge racks

Disposable transfer pipets

P200

Disposable tips, 20-200ul

Clear or white unscented soap

Cups or small beakers

UV lights

BACKGROUND INFORMATION:

General ELISA background information can be found in the preceding lesson, as well as in the BioRad Laboratory Manual to accompany this experiment.

There are several different types of ELISA. For our dengue example, we are indirectly measuring the presence of dengue virus in the patient's serum by capturing antibodies. The steps of an "indirect" ELISA follow the mechanism below:

1. A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
2. A solution of non-reacting protein, such as bovine serum albumin or casein (non-fat milk powder is sometimes used), is added to block any plastic surface in the well that remains uncoated by the antigen.
3. Next the primary antibody is added, which binds specifically to the test antigen that is coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
4. Afterwards, a secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.
5. A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in R&D.
6. The higher the concentration of the primary antibody that was present in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength.

IgM antibody capture ELISA (MAC-ELISA) format is most commonly employed in diagnostic laboratories and commercially available diagnostic kits. The assay is based on capturing human IgM antibodies on a microtiter plate. Dengue virus specific antigen (DENV) is first coated on the plate, followed by the addition of the patient serum sample containing IgM antibodies against dengue (primary antibody). To detect the bound IgM antibodies, anti-human-IgM antibody (the secondary antibody) is added to the plate. The enzyme-linked anti-human antibody will bind to the patient IgM. Once substrate is added, the enzyme is released causing a color change. The antigens used for this assay are derived from the envelope protein of the virus. One of the limitations of this testing is the cross reactivity between other circulating flaviviruses such as West Nile Virus. This limitation must be considered when working in regions where multiple flaviviruses co-circulate. IgM detection is not useful for dengue serotype determination due to cross-reactivity of the antibody. RT-PCR is used to determine the serotype, as covered in the next lesson.

ADVANCE PREPARATION:

All directions for performing the ELISA can be found in the instruction manual which accompanies the BioRad kit. There is advance preparation for use of the kit. For advanced classes, teachers may even consider extending the lesson to include a quantitative analysis. As this unit is written now, there is only one patient to test, and she is positive, as are the other two initial cases reported. Later in the unit, students will analyze results from multiple individuals. The simulation instructions presented here are modeled after the BioRad kit, Protocol III. Therefore, whether the students are performing the authentic BioRad ELISA or a simulation, they will follow the same steps.

1. Prepare the ELISA plates.

If using 12-well microplate strips, use a Sharpie or other permanent marker to number the wells at the top 1-12. If using 96-well plates, they should come with columns and rows marked.

2. Using a fluorescent ink pen, “paint” the bottom of wells 1-3 (positive serum) and wells 7-9 (patient serum). Allow to dry prior to use.

3. Prepare student station reagents using the chart below. Note: This provides quantities for 8 student workstations, each with 2-4 students. To allow students to work in smaller groups, but without increasing prep time aliquoting reagents, two student groups (2 microstrip plates) can use 1 set of reagents.

Tubes (number needed)	Description	Label	Contents (Each Tube)
Violet tubes, 8	Positive controls	+	0.5ml water
Blue tubes, 8	Negative controls	-	0.5ml water
Green tubes, 8	Purified antigen	AG	1.5ml water
Orange tubes, 8	Secondary antibody	SA	1.5ml water
Brown tubes, 8	Enzyme substrate	SUB	1.5ml water
Clear tubes, 8	Patient sample	PAT	0.25ml water

3. Prepare wash buffer

Add 5ml clear or white unscented dish soap to 1000ml water. Mix well.

Aliquot 50ml wash buffer per student group (Beakers, conical tubes, or cups work well.)

4. Assemble student workstations, or have students collect the items below from a common station.

Item (Label)	Contents	Number per station	(<input checked="" type="checkbox"/>)
Yellow tube (PAT)	Patient sample (0.25ml)	1	
Violet tube (+)	Positive control (0.5ml)	1	
Blue tube (-)	Negative control (0.5ml)	1	
Green tube (AG)	Purified antigen (1.5ml)	1	
Orange tube (SA)	Secondary antibody (1.5ml)	1	
Brown tube (SUB)	Enzyme substrate (1.5ml)	1	
Beaker of wash buffer		1	
12-well microplate strip		1	
Disposable transfer pipette		7 (only 1 needed for wash buffer if using P200)	
20-200ul micropipette		1 (if available)	
20-200ul tips		1 box (if available)	
Stack of paper towels		1	

Implementation tips:

- Use P200 if available to add samples to the wells.
- Use disposable pipettes to add the wash buffer.
- Ensure students know how to use both the adjustable volume pipette as well as the disposable pipettes. Bubbles are not friendly in this experiment, and improper use of the pipettors has led to many wells bubbling over.
- Use absorbent towels. The brown paper towel standard in many schools does not adequately absorb liquid, causing samples to splash back and contaminate adjacent wells. This isn't a problem with the simulation, but is with an actual ELISA.
- Avoid rehydrating the antibodies, particularly the secondary antibody, until just prior to use.
- If possible, when performing the actual ELISA, keep all solutions and reagents cold until use.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

The procedure is well written in the BioRad manual.

Read or provide copies of the continuation of case report 1 and remind students to record results in their epidemiological report.

Story cont.

During the patient's third visit on August 17, a consulting infectious-disease specialist raised the possibility of dengue infection, despite no recent travel by the patient to a known dengue-endemic area. However, on the day of illness onset, she had returned from a 1-week trip to Key West, where she had received multiple mosquito bites. A serum sample is sent to a private laboratory to test for exposure to dengue virus. You will now take on the role of a laboratory technologist and perform the ELISA test for antibodies to Dengue virus. Once the test is complete, be sure to record your results in the epidemiological report.

PROCEDURE CONTINUED

Since the patient tested positive for Dengue, the CDC and Monroe County Health Department are notified. They are interested in which serotype of Dengue the patient has. They have requested additional testing. Fortunately, a CSF sample was taken early in the course of the disease which may be able to be used to detect the serotype.

ASSESSMENT SUGGESTIONS:

BioRad includes focus and review questions which can be collected for assessment.

Modifications:

- Could test for different mosquito diseases such as West Nile, Dengue, Yellow Fever
- Could do a mock rapid test rather than ELISA (glucose strips, pH strips?)

EXTENSIONS:

ACTIVITIES:

LITERATURE:

RESOURCES/REFERENCES:

Student ELISA Procedure

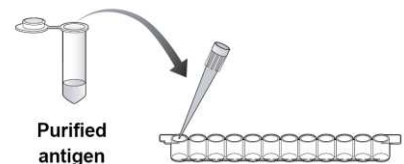
Our story continues.

During the patient's third visit on August 17, a consulting infectious-disease specialist raised the possibility of dengue infection, despite no recent travel by the patient to a known dengue-endemic area. However, on the day of illness onset, she had returned from a 1-week trip to Key West, where she had received multiple mosquito bites. A serum sample is sent to a private laboratory to test for exposure to dengue virus. You will now take on the role of a laboratory technologist and perform the ELISA test for antibodies to Dengue virus. Once the test is complete, be sure to record your results in the epidemiological report.

1. Review the student workstation checklist to ensure you have all needed reagents and supplies.

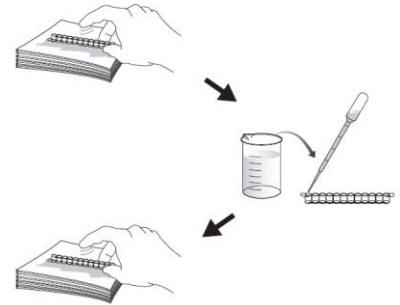
Item (Label)	Contents	Number per station	✓
Yellow tube (PAT)	Patient sample (0.25ml)	1	
Violet tube (+)	Positive control (0.5ml)	1	
Blue tube (-)	Negative control (0.5ml)	1	
Green tube (AG)	Purified antigen (1.5ml)	1	
Orange tube (SA)	Secondary antibody (1.5ml)	1	
Brown tube (SUB)	Enzyme substrate (1.5ml)	1	
Beaker of wash buffer		1	
12-well microplate strip		1	
Disposable transfer pipette		7 (only 1 needed for wash buffer if using P200)	
20-200ul micropipette		1 (if available)	
20-200ul tips		1 box (if available)	
Stack of paper towels		1	

2. Label wells 1-3 with a + (positive); label wells 4-6 with – (negative); label wells 7-9 with Pat (patient).
3. Use a fresh pipette tip to transfer 50ul of the purified antigen (AG) into wells 1-9 of the microplate strip.
4. Wait 5 minutes for the purified dengue virus antigen to bind to the plastic wells.



5. Wash:

- a. Tip the microplate strip upside down onto the paper towels, and tap the strip a few times upside down. Make sure to avoid splashing sample back into wells.
- b. Discard the top paper towel.
- c. Use your transfer pipette to fill each well (1-9) with wash buffer, taking care not to spill over into neighboring wells. Note: the same transfer pipette is used for all washing steps. Be sure to only draw up wash buffer, and not the contents of the wells.
- d. Tip the microplate strip upside down onto the paper towels and tap.
- e. Discard the top 2-3 paper towels.



6. Repeat wash step 4

7. Use a fresh pipette tip to transfer 50ul of the positive control (+) into wells 1-3.

8. Use a fresh pipette tip to transfer 50ul of the negative control (-) into wells 4-6.

9. Use a fresh pipette tip to transfer 50ul of the patient serum (PAT) into wells 7-9.

10. Leave wells 10-12 empty.

11. Wait 5 minutes for the antibodies to bind to their targets.

12. Wash the unbound primary antibody out of the wells by repeating all of wash step 4 two times. (Wash twice.)

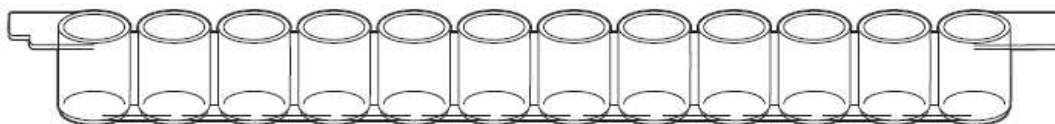
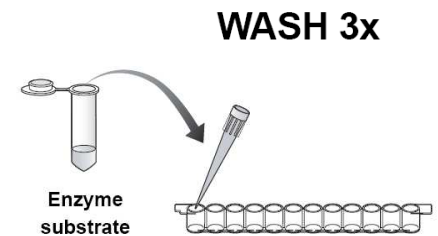
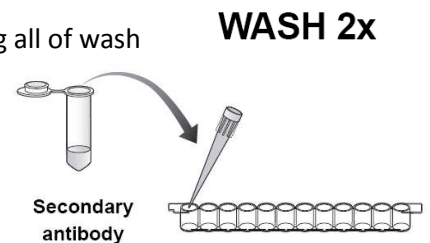
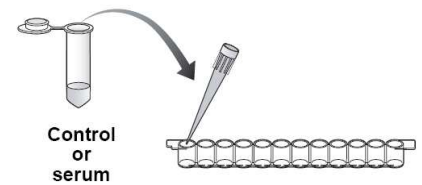
13. Use a fresh pipette tip to transfer 50ul of secondary antibody (SA) into wells 1-9 of the microplate strip.

14. Wait 5 minutes for the antibodies to bind to their targets.

15. Wash the unbound secondary antibody out of the wells by repeating wash step 4 three times. (Wash three times.)

16. Use a fresh pipette tip to transfer 50ul of enzyme substrate (SUB) into wells 1-9 of the microplate strip.

17. Wait 5 minutes. Observe and record the results on your epidemiological report.



LESSON 5: Gel Electrophoresis

KEY QUESTION(S): Can dengue virus be detected using molecular methods?

OVERALL TIME ESTIMATE: 45 minutes

LEARNING STYLES: Visual, kinesthetic and auditory

VOCABULARY:

DNA

RNA

cDNA

Polymerase chain reaction

RT-PCR

Agarose

Gel electrophoresis

LESSON SUMMARY: 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.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

Explain the process of PCR

Know (Describe) what the bands on an agarose gel represent

Explain how gel electrophoresis works

Interpret (and use) the banding pattern on an agarose gel

Compare and contrast the use of PCR vs ELISA

STANDARDS:

MATERIALS:

Dyes:

Xylene cyanol = DENV-1

Ponceau G= DENV-2

Bromphenol blue = DENV-3

Methyl orange= DENV-4

Source	Student workstation	Number required per group	Number required for class
Carolina Biological: Introductory Gel Electrophoresis Teacher Demo Kit Cat # 21-1147 or 8-Station Classroom Kit Cat # 21-	PCR samples (bromophenol blue, methyl orange, ponceau G, xylene cyanol)		

1148			
Classroom laboratory	Sterile distilled H ₂ O, 500µl aliquot	1 tube	
Bio-Rad http://www.bio-rad.com 2–20 µl Digital Micropipet 166-0506EDU, \$221.00 OR Classroom 2–20 µl Digital Micropipet 166-0551EDU, \$110.00	P20 pipette	1	
Fisher Scientific http://www.fishersci.com/ Cat. No. 50863814 Pack of 960 for \$120.99 (10 boxes of tips with 96 tips per box)	P20 pipette tips	1 box	
Fisher Scientific http://www.fishersci.com/	Bromphenol blue		
Fisher Scientific http://www.fishersci.com/	Methyl orange		
Fisher Scientific http://www.fishersci.com/	Ponceau G		
Fisher Scientific http://www.fishersci.com/	Xylene cyanol		
	15ml conical tubes		
	1.5 – 2.0ml microfuge tubes		
Life Technologies/ Invitrogen http://www.invitrogen.com/ E-Gel® 1.2% with SYBR Safe™ Starter Kit Cat. No. G6206-01, \$109.00 (includes PowerBase, adaptor plug, and six 1.2% E-Gels)	E-Gel® PowerBase™ and adaptor plug	1	
Life Technology/ Invitrogen http://www.invitrogen.com/ E-Gel® 1.2% with SYBR Safe™18-Pak Cat. No. G5218-01, \$170.00 (Gels only)	E-Gel® 1.2% with SYBR Safe™	1	

Source	Instructor's (common) workstation	Number required
Bio-Rad http://www.bio-rad.com	Centrifuge (2.0ml tubes)	1

Cat. No. 166-0603EDU, \$299.00		
Fisher Scientific	White light box (optional)	1
Classroom or personal	Digital camera (optional)	1

BACKGROUND INFORMATION:

There are four serotypes of the dengue virus, and it is useful for researchers and health officials to know which type of dengue is circulating to track the movement of the virus and potential severity of disease outbreaks. Some serotypes are more virulent; additionally, infection with multiple serotypes can lead to a severe reaction including dengue hemorrhagic fever.

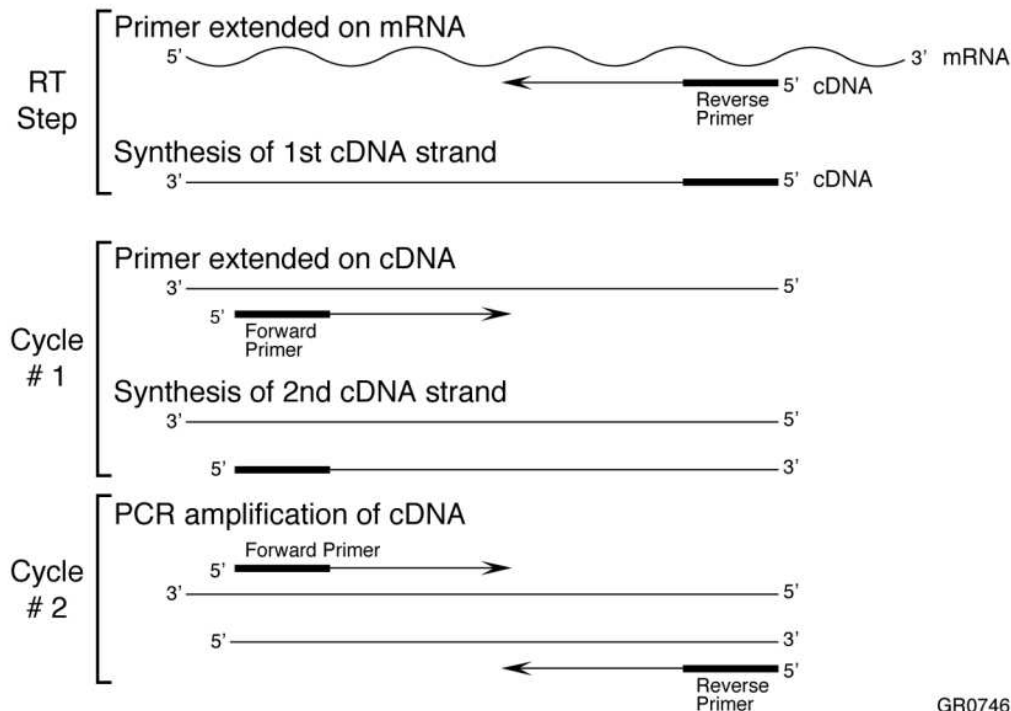
To determine the serotype, a small, unique segment can be amplified using polymerase chain reaction (PCR). Short DNA sequences called primers are designed for each serotype and will only bind and allow amplification of its specific serotype. Dengue virus is an RNA virus. We can still carry out PCR, but first we must perform reverse transcription to create cDNA (complementary DNA) from the RNA virus template. PCR then proceeds as normal using the new cDNA as the template.

To learn more about the process of PCR, view the animation at:

<http://www.sumanasinc.com/webcontent/animations/content/pcr.html>

For Reverse Transcription-PCR, view the animation at:

http://www.bio.davidson.edu/Courses/immunology/Flash/RT_PCR.html



GR0746

Image from Applied Biosystems

Once thousands of copies of the target DNA have been produced, a process called gel electrophoresis is used to separate the DNA fragments. Moving through the agarose gel, DNA fragments will form bands that are visualized by staining the DNA. Comparing our sample to a positive control allows us to measure the size of the DNA fragment and determine which dengue serotype was present in the original sample.

<http://www.dnalc.org/resources/animations/gelectrophoresis.html>

ADVANCE PREPARATION:

1. Order dyes in powder form.

**Alternatively, the Introductory Gel Electrophoresis from Carolina Biological (Teacher Demo Kit Cat # 21-1147 or 8-Station Classroom Kit Cat # 21-1148 can be used.*

2. **Prepare the stock solutions:** Add 0.025g dye to 10ml water. Mix well.

**Take care when mixing. These are very fine powders that become airborne quite easily.*

If using standard gel electrophoresis units, add 1mL glycerol to stock solution.

Powder dye	+	Water	=	Contents (Each Tube)	Label
0.025g Xylene cyanol	+	10mL water	=	10mL stock xylene cyanol (used for DENV-1 positive control and patient sample)	DENV-1 Stock soln
0.025g Ponceau G	+	10mL water	=	10mL stock Ponceau G (used for DENV-2 positive control)	DENV-2 Stock soln
0.025g Bromphenol blue	+	10mL water	=	10mL stock Bromphenol blue (used for DENV-3 positive control)	DENV-3 Stock soln
0.025g Methyl orange	+	10mL water	=	10mL stock Methyl orange (used for DENV-4 positive control)	DENV-4 Stock soln

3. **Prepare dilute (working) solutions:** Mix 1ml stock solution with 3ml water. Mix well.

Stock soln	+	Water	=	Contents (Each Tube)	Label
1mL DENV-1 Stock soln (Xylene cyanol)	+	3mL water	=	4mL dilute xylene cyanol (used for DENV-1 positive control and patient sample)	DENV-1 dilute soln
1mL DENV-2 Stock soln (Ponceau G)	+	3mL water	=	4mL dilute Ponceau G (used for DENV-2 positive control)	DENV-2 dilute soln
1mL DENV-3 Stock soln (Bromphenol blue)	+	3mL water	=	4mL dilute Bromphenol blue (used for DENV-3 positive control)	DENV-3 dilute soln
1mL DENV-4 Stock soln (Methyl orange)	+	3mL water	=	4mL dilute Methyl orange (used for DENV-4 positive control)	DENV-4 dilute soln
1mL each DENV stock solution			=	4mL Multiplex reaction	Multiplex reaction

4. **Aliquot and label student samples:** Each student group will need one of each labeled tube type, i.e., one tube DENV-1, one tube DENV-2, one tube DENV-3, one tube DENV-4, one tube Multi, one tube PAT, one tube Water

Tubes (number needed)	Description	Label	Contents (Each Tube)
1.5mL tubes, 8	Dengue serotype 1 positive control	DENV-1	25µL Xylene cyanol
1.5mL tubes, 8	Dengue serotype 2 positive control	DENV-2	25µL Ponceau G
1.5mL tubes, 8	Dengue serotype 3 positive control	DENV-3	25µL Bromphenol blue
1.5mL tubes, 8	Dengue serotype 4 positive control	DENV-4	25µL Methyl orange
1.5mL tubes, 8	Dengue multiplex reaction control	Multi	25µL Multiplex
1.5mL tubes, 8	Patient sample	PAT	25µL Xylene cyanol
1.5mL tubes, 8	Water	WATER	500µL Water

5. **Set-up lab stations,** or have a common station for students to gather the following:

Student workstation	Number required (per student group)
PCR samples (DENV-1, DENV-2, DENV-3, DENV-4, DENV multiplex, and patient)	1 set (of 6 tubes)
Sterile distilled H ₂ O	1 tube
P20 pipette	1
P20 pipette tips	1 box
E-Gel® PowerBase™ and adaptor plug	1
E-Gel® 0.8% or 1.2% with SYBR Safe™	1
Waste container for tips	1
Gel Electrophoresis Protocol	1
Gel Electrophoresis Student Worksheet	1 per student

*Implementation notes:

- This lesson is written for use with the Invitrogen EGel system. Standard agarose gels (~0.8-1.2%) in either TAE or TBE could be substituted. Voltage and run time will vary with different systems. I have had consistently positive results with ~120 volts for 20 minutes. Watch your gel to make sure it isn't getting too hot and the samples don't run off the gel.
- This is a simulation. The samples for electrophoresis are different dyes that migrate at different rates. They are also different colors. You may wish to offer the explanation that the 3' primer, which is unique for each serotype, also has a dye attached so that we can easily analyze our results without the need for an additional staining step.
- If you use the kit from Carolina Biological, be aware it includes one positively charged dye (Pyronin Y) which you do not want to use for this activity.

- Depending on the depth of background students need, you may want to consider introducing PCR and gel electrophoresis on day one, then starting with the actual gel the following day. On day one, students can also practice pipetting using the CPET activity (See Extension Activities).
- Alternatively, students could be given the worksheet for homework to read and visit the animations prior to class. Then they could start the gel (which should run ~15 minutes, although since it is just dyes, they will start making guesses within the first couple of minutes) and while the gel is running, either review the homework or have a paper version of pcr and/or gel electrophoresis to reinforce the concepts. The PCR song is a great inclusion.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Remind students of general lab safety rules and extra cautions for today. (Working with liquid buffer and electricity. No horse play. Use the equipment only as instructed.)
2. Distribute Student Worksheet.
3. Allow students time to read the worksheet silently.
4. Provide students with an introduction to PCR and gel electrophoresis using the animations. You may wish to spend more time on these topics, but for this lesson, a general knowledge is all that is needed.
5. Tell the students we are returning to our patient case #1. Using the CSF sample taken on August 13th we are going to determine the dengue serotype. (A later lesson will address why a previous sample was used rather than the one from the 17th or a new one. The key is we are trying to detect actual viruses, not the body's immune response.) The sample has already been amplified by RT-PCR and is ready to be run on an agarose gel for analysis.
6. Instruct students to follow the directions on the worksheet.
7. Remind students to record their results on the epidemiological report.
8. Wrap up the lesson by reading the Case # 1 conclusion below.

Both serum specimens (from August 17 and September 3) were positive for dengue IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC ELISA). Dengue virus serotype 1 (DENV-1) was detected by reverse transcription--polymerase chain reaction (RT-PCR) from the CSF specimen taken on August 13. The patient had improved when she returned to her primary-care provider on August 19, and she had completely recovered when interviewed by Monroe County Health Department on September 1.

ASSESSMENT SUGGESTIONS:

An electrophoresis quiz is included as an assessment piece.

EXTENSIONS:

ACTIVITIES: Sort and See, BioRad's PCR Song, PCR Dash (available to borrow from UF CPET)

LITERATURE: Kary Mullis' Nobel Prize Speech

RESOURCES/REFERENCES:

Original PCR paper by Mullis

Typing of Dengue Viruses in Clinical Specimens and Mosquitoes by Single-Tube Multiplex Reverse Transcriptase PCR

This protocol is an adaptation of a two-step nested RT-PCR assay described by Lanciotti et al. (18). Five oligonucleotide primers are included in the one-step assay: one 5' primer that targets a region of the capsid gene conserved in all four dengue virus serotypes and four 3' primers, each of which is complementary to sequences unique to each serotype. These primers are positioned such that a differently sized product is generated from each type, as shown in Fig. 1A, lanes 1 to 4 (dengue-2, 119 bp; dengue-3, 290 bp; dengue-4, 389 bp; dengue-1, 482 bp).

Additional PCR animations:

<http://www.dnalc.org/resources/animations/pcr.html>

<http://highered.mcgraw-hill.com/olc/dl/120078/micro15.swf>

<http://learn.genetics.utah.edu/content/labs/pcr/> (virtual lab)

http://www.bio.davidson.edu/courses/Immunology/Flash/RT_PCR.html (old school animation of RT-PCR)

Gel Electrophoresis Student Worksheet

Testing of a serum specimen at a private laboratory revealed dengue immunoglobulin M (IgM) antibodies. After her physician notified Monroe County Health Department (MCHD) of the test result, the patient's serum specimen from August 17, a cerebral spinal fluid (CSF) specimen from August 13, and a repeat serum specimen from September 3 were sent to CDC for confirmatory testing.

Record information on your epidemiological report.

Activity background information:

There are four different types of dengue virus referred to as serotypes, and it is useful for researchers and health officials to know which type of dengue is circulating to track the movement of the virus and potential severity of disease outbreaks. Some serotypes are more virulent; additionally, infection with multiple serotypes can lead to a severe reaction including dengue hemorrhagic fever.

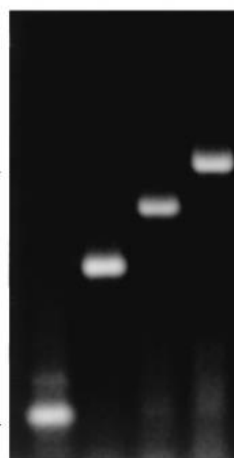
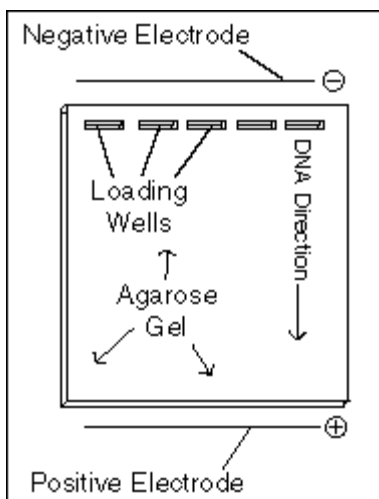
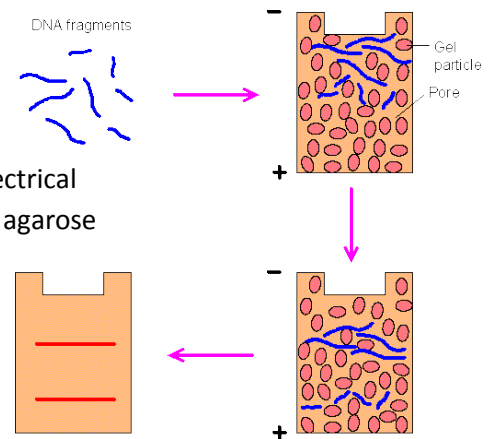
To determine the serotype, a small, unique segment can be amplified using polymerase chain reaction (PCR). Short DNA sequences called primers are designed for each serotype and will only bind and allow amplification of its specific serotype. Dengue virus is an RNA virus. We can still carry out PCR, but first we must perform reverse transcription to create cDNA (complementary DNA) from the RNA virus template. PCR then proceeds as normal using cDNA as the template.

To learn more about the process of PCR, view the animation at:

<http://www.sumanasinc.com/webcontent/animations/content/pcr.html>

Once thousands of copies of the target DNA have been produced, a process called gel electrophoresis is used to separate the DNA fragments. Since all DNA is negatively charged, when we place the fragments in an electrical field, the smaller pieces will be able to travel farther. Moving through the agarose gel, DNA fragments will form bands that are visualized by staining the DNA. Comparing our sample to a positive control, allows us to determine which dengue serotype was present in the patient sample.

<http://www.dnalc.org/resources/animations/gelectrophoresis.html>



DENV1: 482bp

DENV4: 389bp

DENV3: 290bp

DENV2: 119bp

54 Dengue

DNA Gel Electrophoresis Laboratory

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab:

Student workstation	Number required
PCR samples (Positive controls: DEN1, DEN2, DEN3, DEN4, DEN multiplex, patient)	6
Sterile distilled H ₂ O	1 tube
P20 pipette	1
P20 pipette tips	1 box
E-Gel® PowerBase™ and adaptor plug	1
E-Gel® Precast Agarose Gel	1
Waste container for tips	1

Laboratory Method for DNA Gel Electrophoresis using the E-Gel System

Prepare gel

1. Plug PowerBase™ into an electrical outlet.
2. Remove gel cassette from package.
3. Insert the gel (with comb in place) into the base right edge first. Do this by tilting the gel and placing the right edge in first, under the electrode cover. Once the right side of the gel is placed, the left side can be snapped into place. **Do not press straight down on the gel or the electrodes will be damaged.** The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.

Load prepared samples

1. Remove and discard comb from the E-Gel® cassette.
2. Add 20µl sterile distilled H₂O to wells 1-3 and 10-12.
3. Add 20µl PCR samples to wells 4-9 (DENV Controls, Patient Case #1).

Well #	1	2	3	4	5	6	7	8	9	10	11	12
DNA	--	--	--	20µl DENV1 control	20µl DENV2 control	20µl DENV3 control	20µl DENV4 control	20µl DENV multiplex control	20µl Patient 1	--	--	--
H ₂ O	20µl	20µl	20µl	--	--	--	--	--	--	20µl	20µl	20µl

Run gel

1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.
3. Remove the gel cassette. Lift up the left side first, and then slide out toward the left while still angled to avoid damaging the electrodes. **Do not pull the gel straight up to remove.**
4. Analyze your results by viewing on a piece of white paper.
5. Sketch a picture of your gel on the Epidemiological Report.

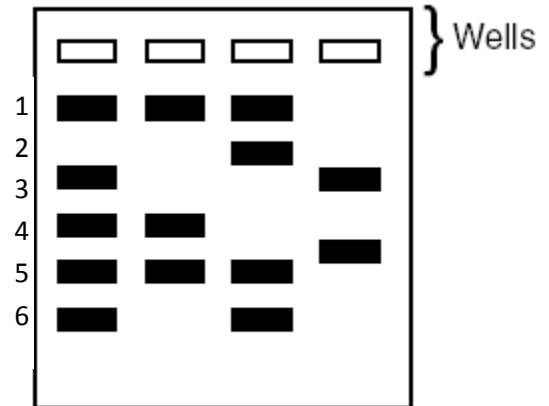
Gel Electrophoresis Quiz

1. Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? _____ Why do the DNA molecules move toward this pole?
2. Describe how different sized DNA fragments are separated by the agarose gel matrix.
3. Examine the diagram of an agarose gel and answer the following questions.
 - a. What do the bands in the drawing of the agarose gel represent?

b. Which band(s) contain the largest fragments of DNA?

c. Which band(s) contain the smallest fragments of DNA?

d. On the drawing, label the positive and negative ends of the gel.



Gel Electrophoresis Quiz – Answer Key

1. Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? _____ Why do the DNA molecules move toward this pole?

DNA moves toward the positive pole due to its negative charge. The phosphate groups of the DNA backbone confer an overall negative charge to the DNA molecule.

2. Describe how different sized DNA fragments are separated by the agarose gel matrix.

All DNA is negatively charged and all will move toward the positive electrode. The smaller pieces of DNA can snake through the pores in the gel, moving faster through the gel. The smaller pieces will be at the bottom of the gel.

3. Examine the diagram of an agarose gel and answer the following questions.

- a. What do the bands in the drawing of the agarose gel represent?

Many fragments of same-size DNA co-migrating

- b. Which band(s) contain the largest fragments of DNA?

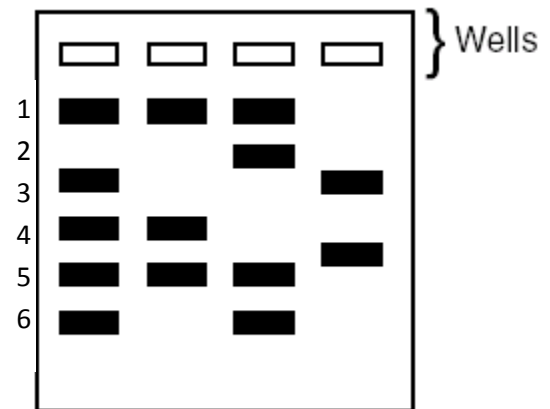
1

- c. Which band(s) contain the smallest fragments of DNA?

6

- d. On the drawing, label the positive and negative ends of the gel.

Negative end by the wells; positive at the bottom.



LESSON 6: Different Tests for Different Stages

KEY QUESTION(S): Why are different molecular tests used to diagnose dengue virus?

OVERALL TIME ESTIMATE: 30 minutes

LEARNING STYLES: Visual

VOCABULARY:

ELISA

RT-PCR

Acute

Viral load

Viremia

LESSON SUMMARY: 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.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

Compare and contrast ELISA and RT-PCR

Understand the difference between antibodies (proteins) and DNA and know which test uses each macromolecule

Interpret a graphical model of immune response

Understand viral load is highest at the on-set of an illness and decreases as the body's immune system responds to the infection

STANDARDS:

MATERIALS:

Student worksheet (one per student)

BACKGROUND INFORMATION:

As with most infections, the titers of the infectious agent are usually highest at the on-set of the infection, before the host immune response. Once the host immune system is able to launch a response, antibodies to the infectious agent begin circulating and neutralizing the foreign antigens. In the case of dengue virus, this corresponds to a rapid increase in virus particles immediately after exposure. During the early stages of disease the virus titer is still high, but declining as the immune response begins. During the first ~5 days of disease on-set, dengue virus can be detected using RT-PCR. Once the dengue virus titer drops to practically zero, it can no longer be detected by nucleic acid means (RT-PCR) but patient serum IgM antibodies are now circulating and can be assayed using ELISA. When symptoms have resolved, IgM levels drop, but IgG remains present, waiting for the next infection. During a secondary infection, IgG will spike quickly, trying to neutralize the infection dengue virus, but

antibody enhancement can actually cause a more severe secondary reaction, leading to dengue hemorrhagic fever.

The graph includes the NS1 ELISA. The non-structural protein 1 (NS1) of the dengue viral genome has been shown to be useful as a tool for the diagnosis of acute dengue infections. Dengue NS1 antigen has been detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO. The NS1 ELISA based antigen assay is commercially available for DENV and many investigators have evaluated this assay for sensitivity and specificity. The NS1 assay may also be useful for differential diagnostics between flaviviruses because of the specificity of the assay. The MAC-ELISA is the most commonly used assay for dengue, and to avoid confusion, is not formally introduced in this unit.

ADVANCE PREPARATION:

Make copies of student worksheet (1 per student)

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

30 minutes

1. Review with the students the previous activities asking what they were testing for in each:
 - ELISA: testing patient serum for the presence of antibodies against dengue. This is an antibody (or protein) based assay.
 - RT-PCR: testing patient spinal fluid for the presence of dengue virus. This is a nucleic acid based test.
2. Ask the students to recall a time they had a cold.
3. Call on students to discuss how the cold progressed, specifically symptoms and how their immune system responded. They don't need to know specific cell types and antibodies, (although if they have already covered this material, this is an excellent review opportunity), just generally what is going on in their body when they are "fighting a cold".
4. **Help lead the students to the following ideas: (stages of cold = symptoms)**
 - There are thousands of different cold viruses (rhinoviruses), which is why we get sick so often. Our bodies don't have immunity to them all.
 - The virus was able to rapidly multiply and spread within the body since it was a new virus.
 - The human body tried to combat the infection with its first line of defense: fever, chills. Fatigue is a symptom of the body fighting.
 - First response kills some of the virus causing the virus titer (numbers) to begin to drop.
 - The secondary line of defense is ramped up and infected cells are tagged and destroyed.
 - Antibodies are generated, seeking out the invading particles and neutralizing them.
5. Distribute the student worksheet.
6. Tell the students this graphical model is specifically for dengue, but many infectious agents cause a similar response. Remind them that biological systems are dynamic and don't always conform to exact timelines. Therefore, this represents the average response.
7. Allow students time to work in class or assign as homework.

ASSESSMENT SUGGESTIONS:

Worksheet can be collected.

MODIFICATIONS:

For advanced students, print out or direct students to [Host Response to the Dengue Virus](#). This article provides a good explanation of the immune response specifically to dengue virus and also secondary dengue infections. This information will help when discussing vaccine development.

EXTENSIONS:

ACTIVITIES: Have students act out immune system role play

<http://mypages.iit.edu/~smile/bi9212.html>

<http://peer.tamu.edu/LessonPlan.asp?id=128&file=activity>

<http://www.lessonplanet.com/search?keywords=immune+response+role+play&media=lesson>

HHMI Click and Learn: http://www.hhmi.org/biointeractive/disease/immunology_primer/01.html

Make posters or models of key immune system players

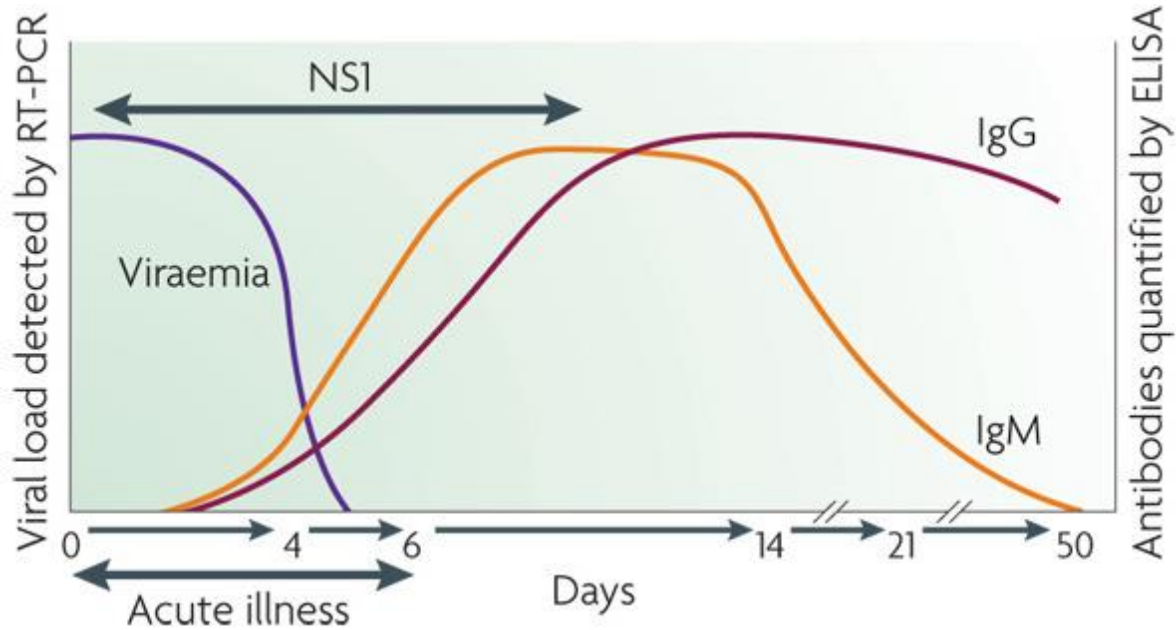
LITERATURE:

Host Response to the Dengue Virus (from Nature Education's Scitable)

<http://www.nature.com/scitable/topicpage/host-response-to-the-dengue-virus-22402106>

RESOURCES/REFERENCES:

Answer the following questions using the graph below.



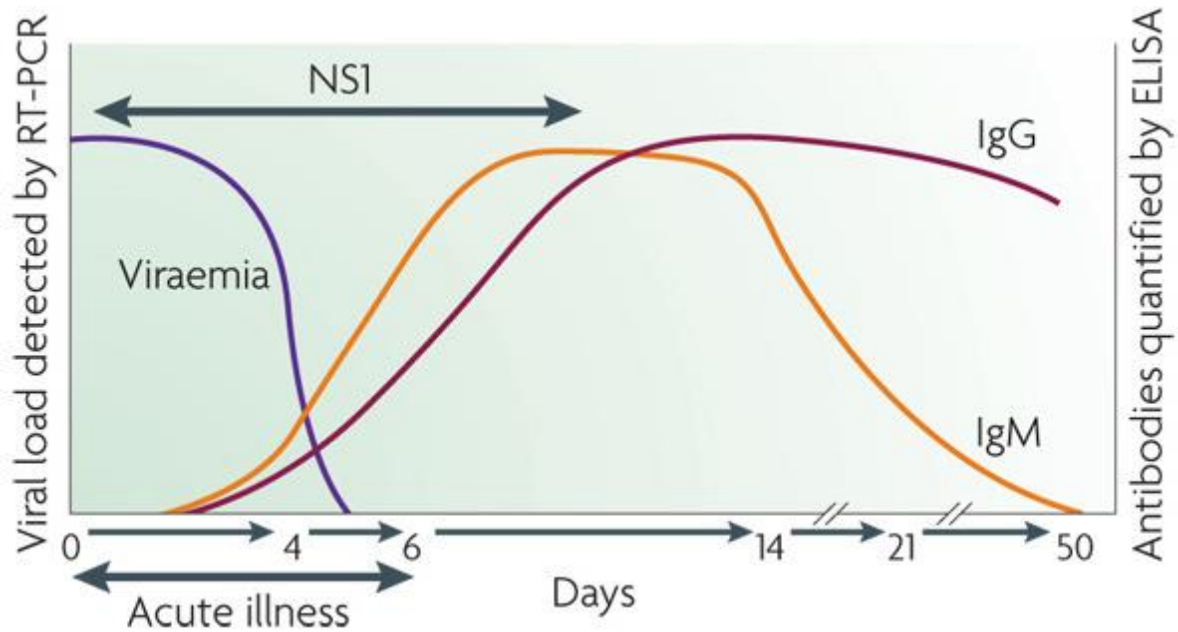
<http://www.nature.com/scitable/topicpage/host-response-to-the-dengue-virus-22402106>

1. When is the level of IgM highest? _____
2. What test would you use to detect serum antibodies? _____
3. When is the last day you can detect virus in a patient sample? _____
4. What test is used to detect viraemia? _____
5. How long does the acute illness last? _____
6. Explain why the viral load is high during early days, but drops rapidly. _____

7. Why can't we use an ELISA during the acute phase of the illness? _____

8. Now consider our patient case #1. Review her epidemiological report and estimate the dates of her acute illness by comparing the patient symptoms and testing timeline with this graph.

Answer Key



<http://www.nature.com/scitable/topicpage/host-response-to-the-dengue-virus-22402106>

1. When is the level of IgM highest? ~days 7-12
2. What test would you use to detect serum antibodies? MAC ELISA or ELISA
3. When is the last day you can detect virus in a patient sample? Day 5
4. What test is used to detect viraemia? RT-PCR
5. How long does the acute illness last? ~6 days
6. Explain why the viral load is high during early days, but drops rapidly. The virus infects the white blood cells which are destroyed by the body during the immune response. It takes several days for the antibodies in the human to be at a high enough titer to overtake the invading virus.
7. Why can't we use an ELISA during the acute phase of the illness? ELISA measures antibodies or antigens. The viral load isn't high enough during the early phase for detection by ELISA since it drops shortly after infection. During the acute phase, the body also hasn't developed enough circulating antibodies to detect, particularly IgM. For subsequent infections, patients can be screened for IgG which is present much sooner in secondary dengue infections.

LESSON 7: Investigating in the Field

KEY QUESTION(S): How far reaching is dengue virus? Are there people in the community who have been infected but not diagnosed? What type(s) of dengue are in the population? What mosquitoes are present and where?

OVERALL TIME ESTIMATE: 60 minutes (45 minutes day one, 15 minutes day two)

LEARNING STYLES: Visual and kinesthetic

VOCABULARY:

serosurvey

LESSON SUMMARY: Students will assume the role of field epidemiologists as they take to the streets of Old Town in Key West. Randomly selected residences have been interviewed and asked to complete a health survey. Those suspected of dengue infection were asked to give a serum sample for testing. Also, mosquito larvae and adult samples have been collected from the town. They need to be identified to see if there are carriers in the area, where they are located, and then typed for dengue virus to determine what serotype(s) are circulating in the community.

STUDENT LEARNING OBJECTIVES WITH STANDARDS:

The student will be able to...

Interpret ELISA results

Interpret RT-PCR/ gel electrophoresis results

Identify locations on a map

Determine the prevalence of dengue virus in Old Town

Determine the source of the initial infection

MATERIALS:

BACKGROUND INFORMATION:

ADVANCE PREPARATION:

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Divide students into 8 groups.
2. Distribute one field packet to each group. (Currently in ppt format. If have access to laptops in the classroom, these could be used.)
3. Tell them they are to analyze the information in the packets to determine where cases of dengue have occurred in Old Town. Using the report forms, they will compile the data and then indicate on the class map where infected mosquitoes and/or people are.
4. Once all groups have placed their information on the class map, call on each group to share their findings, correcting any misinformation (such as misidentification of mosquitoes, incorrect interpretation of ELISA/PCR results).
5. Ask the students if there seem to be any connections between the source of dengue mosquitoes and positive human cases. (Mosquitoes don't fly far.)

6. Ask the students for their thoughts on what, if anything, should be done about dengue in Key West.
7. Help the students arrive at the idea that if the mosquito life cycle is disrupted, it breaks the chain of transmission. If the HHMI Holiday Lecture or the Dirty Jobs episode were used, draw on those to prompt the students. Dumping standing water. Chemical larvacides and insecticides can be used. There are also biological control methods such as stocking ponds with mosquito fish and *Bacillus thuringiensis* (Bt). Additionally, the community needs to be educated to help reduce their risk of contracting dengue by avoiding contact with mosquitoes. Public education campaigns are common.
8. For homework, ask the students to respond to the writing prompt.

Day 2 (15 minutes)

1. Ask for volunteers to summarize their plan to limit the spread of dengue. (different method for sharing.)
2. Read aloud (or have student volunteers read) the conclusion of the case report- **CASE CLOSED**.
3. Ask if the students agree with what was done. Would they have done anything differently?
4. Did anyone think of a vaccine? If not, ask them if they think this would be possible.
5. In the next short lesson, dengue vaccine development is covered. Could give paper as reading to be done for homework?

ASSESSMENT SUGGESTIONS:

Using the data collected from their section, students can write a field report summarizing their findings. The writing prompt can be collected and graded according to the FCAT Writes rubric.

EXTENSIONS:

ACTIVITIES:

Revisit list from beginning. Reflect on what they have learned, misconceptions corrected, etc.

Create a public service announcement for communities with Dengue outbreaks to reduce mosquito populations and avoid human-mosquito contact. Students could be divided into groups to target specific age groups (i.e., elementary age, middle school, high school, adults, elderly)

LITERATURE:

RESOURCES/REFERENCES:

Teacher Pages

Case 2. On August 31, 2009, Rusty Smith aged 48 years from Key West who reported no recent travel outside Florida went to a clinic with a febrile illness that began August 25. The fever was accompanied by headache, myalgia (muscle pain), joint pain, vomiting, and a truncal maculopapular rash. The patient was diagnosed with a viral syndrome and instructed to return to the clinic in 2 days. He returned on September 2, at which time he requested diagnostic testing for dengue because he had learned of possible dengue transmission in the area. ~~Testing of a serum specimen at a private laboratory identified dengue IgM antibody. Serum from this specimen and a repeat specimen obtained on September 23 were positive at CDC for dengue IgM by MAC ELISA. All of the man's symptoms, except for minor fatigue, resolved and his hemoglobin and platelet counts normalized by September 15.~~

Case 3. While following up on the second case, a nurse at Monroe County Health Department learned that the patient's wife, Abigail Smith aged 46 years, had a similar febrile illness beginning on September 9. Her symptoms included headache, eye pain, pruritic truncal rash (itchy belly rash), nausea and vomiting, chills, and abdominal pain. ~~A diagnosis of dengue subsequently was confirmed by CDC with detection of dengue IgM in a serum specimen by MAC ELISA.~~

Case 4. Calvin Gardiner is 42 and has been living in the Old Town section of Key West his entire life. He owns and works in a souvenir store near the Seaport Boardwalk. He reported having the flu some time during the previous summer, with a high fever, muscle and joint pain, and headache. He remembered being sick for about 3 days.

Case 5. Joe Brightman is 45 and has been living in Old Town for 10 years. He runs a fishing charter boat. He had flu-like symptoms in July: fever, headache, nausea and vomiting. His symptoms lasted for 5 days.

Case 6. Margaret Proctor is the manager of the Best Western motel near where the Cow Key Channel enters the Atlantic Ocean. In early September, she had a fever, and severe headache with nausea and vomiting. She developed a rash on her legs, and had to miss work for 10 days. She is 32 years old.

Case 7. Manuel Perez works for the Keys Film Commission and had persistent headaches during the month of October. He also had a sore throat, cough, and aching neck. He is 25.

Case 8. Tarisha Brown, 24, works in the Hemingway Home and Museum. She reported having a mild case of the flu for about 3 days in July. She had a fever, aching muscles, and fatigue.

Case 9. Gordon Bramston, 31, operates a ferry that travels between Key West, Marco Island, and Fort Meyers. He reported feeling sick in September, with fever, headache, and nausea. He thinks he was sick for about 2 days.

Case 10. Irene O'Connor is a 23 year old mother of two living in the Bahama Village area of Key West. She reported having a fever, headache, and pain in her joints and muscles in August. She was tired and weak for several days after the worst of the symptoms went away.

Case 11. Bethany O'Connor, 11 months old, became sick two days after her mother. Bethany was taken to the pediatrician when her fever reached 101°F. Her grandmother reported that Bethany either just cried or slept for three days, not wanting to be fed or to play.

Ovitrap: An ovitrap is a device which consists of a black cylinder with a piece of cardboard. This device is used to control the *Aedes* mosquito population.

The black ovitrap attracts female mosquitoes to lay their eggs. When the eggs hatch and develop into adults, they cannot fly out of the device and die inside the trap. The extensive use of the ovitrap in a community can be used in *Aedes* population control and effectively reduce the *Aedes* population in that area.

Stickers (spots) of different colors to place on map to indicate presence of carrier species found, positive for DENV (either people or mosquito)

Need 8 sets of data. 8 sections of the town? Each with multiple collection points? To reflect actual numbers: only 10% of patient samples (serosurvey as well as records from docs) were positive (26 of 261). Only 0.5% of mosquito pools were positive (2 of 407).

Maps: http://www.fla-keys.com/brochures_and_maps.cfm

One of old town, keys, south fl

For each group:

Mosquito Patrol

Map location	Evidence of mosquitoes?	Type of container	Mosquito identification	Carrier of dengue?	DENV result

Serosurvey

Map location	Symptoms of dengue?	When did symptoms appear?	Did subject receive medical attention?	DENV result

Case Closed

In response to the three cases of locally acquired dengue, the Florida Keys Mosquito Control District (FKMCD) increased the frequency of truck and aerial spraying to control adult mosquito populations and initiated an intense door-to-door campaign to find and eliminate mosquito breeding sites. Larvicide and handheld adulticide foggers were used when mosquitoes and larvae were found, and ovitrapping and collection of adult mosquitoes was enhanced. During September--December 2009, a total of 407 pools of adult female *Aedes aegypti* mosquitoes from throughout Key West were collected and tested for dengue by PCR at FDOH. Two mosquito pools collected in mid-October tested positive for DENV-1. Testing of mosquito pools in Key West for the presence of dengue is ongoing, and FKMCD and CDC also are testing *Ae. aegypti* mosquitoes in Key West for evidence of insecticide resistance. A public education campaign was conducted by MCHD and FKMCD to emphasize the importance of eliminating mosquito breeding sites and to encourage personal prevention measures against mosquito bites. In addition, FDOH and CDC are providing physician education in south Florida regarding the early identification, prevention, and treatment of dengue.

To determine the extent of dengue infection in the Key West community, a serosurvey was conducted by FDOH and CDC, using randomly selected households, during September 23--27, 2009. Of 240 participants tested, 13 (5.4%) had evidence of recent dengue infection. In addition, Key West physicians were contacted by MCHD and asked to send serum specimens to CDC from all patients with signs and symptoms consistent with dengue. Of 21 specimens submitted during September 23--November 27, nine (42.9%) were positive by either dengue RT-PCR (three), NS-1 assay (one), or IgM ELISA (five). For additional case finding, medical records from three acute health-care facilities in Key West were reviewed for patients treated during July 15--September 15 who had symptoms consistent with dengue infection. Of six persons considered to have dengue-like illnesses and contacted for testing, four were positive for recent dengue infection. Because two of the four cases also had been counted in the serosurvey, the total number of dengue cases acquired in Key West in 2009 was 27, including the index case in the traveler from New York and the 26 cases in Key West residents.

Onset dates in the 27 Key West residents ranged from July 22, 2009, to April 5, 2010 (Figure), indicating that transmission began occurring before the August 10, 2009, onset of symptoms in the New York resident and continued for months afterward. The 28 patients ranged in age from 15 to 73 years (median: 47 years). Fever was reported by all 28; headache, myalgia, arthralgia, eye pain, and rash also were commonly reported (Table). Six patients reported some type of bleeding; four had blood in their urine, two reported gingival bleeding, one reported excessive vaginal bleeding, and one reported epistaxis.

LESSON 8: The Vaccination Quandary

KEY QUESTION(S): Is it possible to develop a vaccine against dengue virus?

OVERALL TIME ESTIMATE:

LEARNING STYLES: Visual and auditory

VOCABULARY:

LESSON SUMMARY: webquest?

<http://www.historyofvaccines.org/>

1. Learn how a vaccine is made. – NOVA
2. Special infection with dengue
3. Think about what the potential problem presents with dengue – which serotype to vaccinate against? How do you do all four- get right combo / dosage to launch immune response / protection without causing harm either during vaccination or with subsequent infection?
4. Current research – clinical trials, papers
5. Dengue hemorrhagic fever and dengue shock syndrome – how do they occur? Who is at greatest risk? Then what is a vaccine? How is it made? Stop. Classroom discussion over the questions: can a vaccine be developed? Allow them to share their ideas in a Socratic method without correcting. Ask them to write down their final answer. Then proceed with rest of webquest – papers, clinical trials. Now answer if think a vaccine can be developed.

STUDENT LEARNING OBJECTIVES WITH STANDARDS:

The student will be able to...

MATERIALS:

BACKGROUND INFORMATION:

ADVANCE PREPARATION:

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

Read scitable paper the night before.

ASSESSMENT SUGGESTIONS:

EXTENSIONS:

ACTIVITIES:

LITERATURE:

RESOURCES/REFERENCES:

http://www.hhmi.org/biointeractive/disease/immunology_primer/01.html

vaccine development articles: <http://www.sciencedaily.com/releases/2011/12/111221151713.htm>

vaccine clinical trial

<http://www.niaid.nih.gov/news/newsreleases/2010/Pages/denguevaxtrial.aspx>

<http://www.niaid.nih.gov/news/newsreleases/2010/Pages/biodefenseVax.aspx>

<http://clinicaltrials.gov/ct2/show/NCT01436422?term=dengue+fever+and+niaid&recr=open&rank=1>

<http://www.tropika.net/svc/review/Anderson-20100413-Review-dengue-vaccine>

Career Spotlight

Throughout the unit, students have taken on different career roles. This lesson investigates those and associated careers a bit closer as the students.

Epidemiologist:

<http://www.bls.gov/ooh/life-physical-and-social-science/epidemiologists.htm>

Medical Technologist:

<http://www.bls.gov/ooh/healthcare/medical-and-clinical-laboratory-technologists-and-technicians.htm>