



The Dengue Dilemma

UF Center For Precollegiate Education & Training

UF UNIVERSITY of
FLORIDA

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Lesson Three Adapted from BioRad

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

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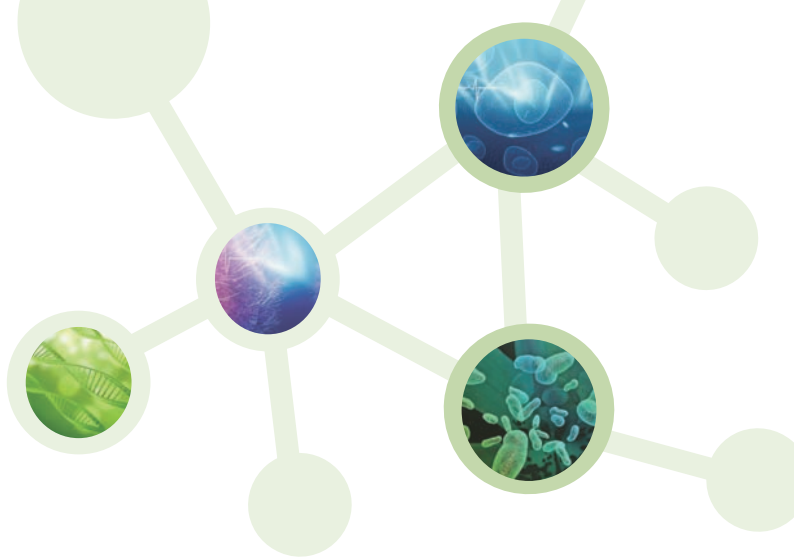
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Introduction

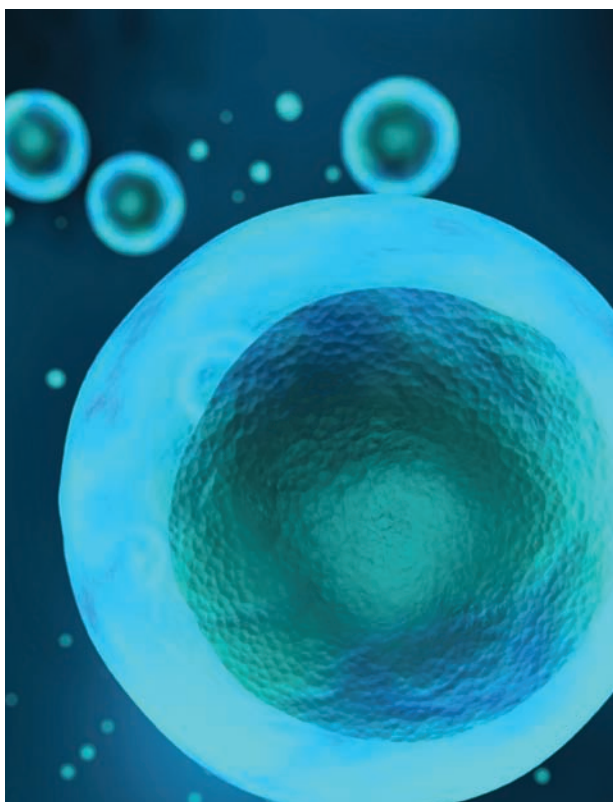
Admittedly, I find pathogens intriguing. In particular, dengue is one of the more interesting. Here is this tiny RNA virus that has four different forms, and can either cause minor discomfort or trigger excruciating pain and even death. It does not exist on its own, but is dependent on a female mosquito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

Author's Note

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

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

Tips About this Curriculum



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

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

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

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

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

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

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

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

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

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

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

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


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

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

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

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

TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.



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

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

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

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

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

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

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

Science Subject: Biology

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

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

Icon Key



Teacher Pages



Student Handout/
Worksheets



Extension



Teacher
Answer Key



Student Reading

Lesson Summaries

LESSON ONE:

What Ails You?

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

LESSON TWO:

Steps of an ELISA

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

LESSON THREE:

Testing for Dengue Antibodies

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

LESSON FOUR:

Gel Electrophoresis

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

LESSON FIVE:

Different Tests for Different Stages

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



Lesson Sequencing Guide

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

	Day 1	Day 2	Day 3	Day 4	Day 5
Week 1	Lesson 1 What Ails You? (45 minutes)	Lesson 2 Steps of an ELISA (45 minutes)	Lesson 3 Testing for Dengue Antibodies (45 minutes)	Lesson 4 Gel Electrophoresis (45 minutes)	Lesson 5 Different Tests for Different Stages (45 minutes)

Vocabulary

Acute: sudden onset of disease; short duration

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

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

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

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

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

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

Endemic: restricted to a particular location or region

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

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

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

Primary Antibody: in ELISA, the first antibody bound

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

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

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

Viremia: presence of viruses in the blood



Next Generation Sunshine State Standards – Science

Standard	Lesson				
	1	2	3	4	5
<p>SC.912.L.14.52 Explain the basic functions of the human immune system, including specific/ nonspecific immune response, vaccines, and antibiotics.</p>	X	X		X	X
<p>SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.</p>	X	X	X		X
<p>SC.912.L.16.11 Discuss the technologies associated with forensic medicine and DNA identification, including RFLP analysis.</p>			X	X	X
<p>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).</p>			X	X	
<p>SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.</p>	X	X	X	X	X
<p>SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.</p>					X
<p>SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.</p>	X			X	
<p>SC.912.N.4.1 Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.</p>				X	
<p>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.</p>					X

Background Information:

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

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

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

The Virus

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

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

Transmission

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

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

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

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



Characteristics

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

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

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

Treatment

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

Prevention

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

Vaccination

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

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

From the WHO Fact Sheet: <http://www.who.int/mediacentre/factsheets/fs117/en/index.html>

From the WHO <http://www.who.int/denguecontrol/en/index.html>

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

Jorge R. Rey from the University of Florida Institute of Food and Agricultural Science has written fantastic pieces that have served as primary background sources and are freely available at: <http://edis.ifas.ufl.edu/in699> (What is Dengue?).

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

What Ails You? The Investigation Begins

1

Vocabulary:

- Dengue fever
- Endemic
- Epidemiology

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 a patient's 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...

1. Interpret a case report
2. Articulate that investigating a disease cause is a detailed process
3. Identify some of the intricacies in investigating a disease cause
4. Recognize it can be difficult to diagnosis an illness

Florida Next Generation Sunshine State Standards (Science):

SC.912.L.14.52 SC.912.L.16.10
SC.912.L.18.1 SC.912.N.3.5

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.

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

🕒 **TIME ESTIMATE:**
• 30 minutes

🧠 **LEARNING STYLES:**
• Visual and auditory

Advance Preparation:

1. Copies of the case report per student.
2. Copies of the epidemiological log per student (Students can work together to fill in their charts, but they will refer to these over the course of the week, so it is helpful for each student to have his/her own.)

Procedure and Discussion Questions with Time Estimates:

30 minutes

1. Distribute a copy 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.

Teaching tip: The students do not have all of the information in this initial report. Remind the students that patients are not always forthcoming with information and this can hinder diagnosis. Additionally, the nurse or doctor who took the patient history may not have asked appropriate probing questions to solicit needed information. Also, while some students may be knowledgeable of dengue, they can only use the information that is actually presented to them rather than filling in the blanks or guessing.

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.

Resources/References:

The Centers for Disease Control has a wealth of information related to dengue including interactive maps and tutorials. <http://www.cdc.gov/dengue/>

This case report is based on the actual first case reported in the 2009/2010 outbreak. For the detailed account of the first three cases, visit: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5919a1.htm>



The Investigation Begins – Case Report

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/ μ L (normal: 4,500–10,500/ μ L), a normal hematocrit of 43%, and a low platelet count of 115,000/ μ L (normal: >150,000/ μ L). 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.

The Investigation Begins – Case Report

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"/> Fever <input type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed Other: _____				
	<input type="checkbox"/> Chills <input type="checkbox"/> Fever <input type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed Other: _____				
	<input type="checkbox"/> Chills <input type="checkbox"/> Fever <input type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed Other: _____	Recovered			
	<input type="checkbox"/> Malaise <input type="checkbox"/> Nausea/vomiting <input type="checkbox"/> Pain behind eyes <input type="checkbox"/> Rash <input type="checkbox"/> Swelling				

Draw or affix image of ELISA below.



Draw or affix image of PCR/gel electrophoresis on back.



The Investigation Begins – Case Report

EPIDEMIOLOGICAL REPORT

Patient Case #: 001 Gender: Male Female Age: 34

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

DATE	SYMPTOMS	SAMPLE SOURCE	TEST PERFORMED	RESULT	DIAGNOSIS
08/11	<input checked="" type="checkbox"/> Chills <input checked="" type="checkbox"/> Fever <input checked="" type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed Other:	Urine	Urine analysis	Bacteruria, hematuria	Urinary tract infection
08/13	<input type="checkbox"/> Chills <input type="checkbox"/> Fever <input checked="" type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input checked="" type="checkbox"/> Light-headed Other:	Lumbar puncture = cerebral spinal fluid	RT-PCR	Positive for DENV-1	On 08/13 – unknown Later testing revealed Dengue fever serotype 1.
08/17	<input type="checkbox"/> Chills <input type="checkbox"/> Fever <input type="checkbox"/> Headache <input type="checkbox"/> Joint/muscle pain <input type="checkbox"/> Light-headed Other:	Serum (blood)	MAC ELISA	Positive for dengue IgM antibodies	On 08/17 – Dengue suspected. Testing confirmed diagnosis.
09/3	Recovered	Serum (blood)	MAC ELISA	Positive for dengue IgM antibodies	Recovered. Circulating dengue antibodies.



Steps of an ELISA

2

Vocabulary:

- Antibody
- Primary antibody
- Antigen
- Secondary antibody
- ELISA

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...

1. Sequence steps of an ELISA test
2. Define ELISA
3. Explain the use of an ELISA to aid in disease diagnosis
4. Describe antigen/antibody interaction
5. Diagram antigen/antibody interaction

Standards:

SC.912.L.14.52 SC.912.L.16.10 SC.912.L.18.1

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.

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

🕒 TIME ESTIMATE:
• 15 – 45 minutes depending on prior knowledge

🎯 LEARNING STYLES:
• Visual, kinesthetic and auditory

Advance Preparation:

- Project the image of antigens and antibodies.
- Teaching tip: Make this into a file folder game. Affix the Steps of an ELISA student worksheet to the inside left and right sides of a file folder. Laminate for repeated use. Color copy the ELISA cards, laminate, and cut. Using small pieces of Velcro, place one side on the ELISA cards and the other in the center of each step on the worksheet.

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 gray 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.

Resources/References:

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



Steps of an ELISA

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.

	<p>Virus proteins (antigens) are added to wells of a 96-well plate.</p>
	<p>The antigens bind to the plastic, coating the bottom of the wells.</p>
	<p>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.</p>
	<p>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>



	<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>



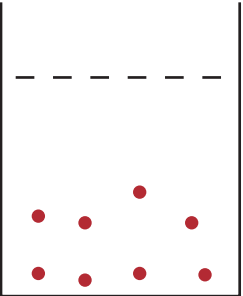
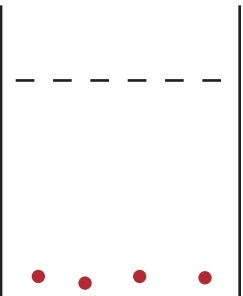
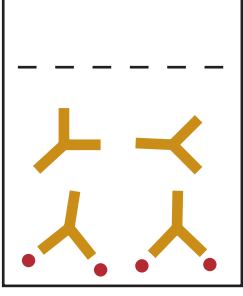
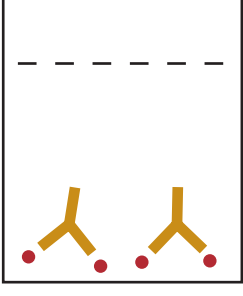
Make one set of eight cards for each group. Cut along dotted lines to separate into eight cards, each representing a step in the ELISA reaction.

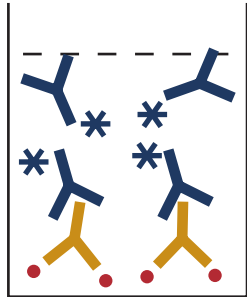


Steps of an ELISA

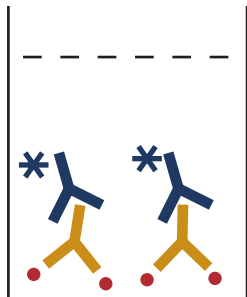
A common test used to detect if a patient has been exposed to a virus such as HIV, Dengue, or West Nile is called an ELISA (Enzyme Linked ImmunoSorbant Assay). This test takes advantage of the interactions between antigens and antibodies. Often compared to a lock and key, an antigen/antibody interaction 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.

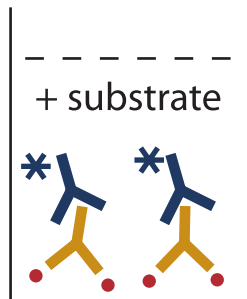
	<p>Virus proteins (antigens) are added to wells of a 96-well plate.</p>
	<p>The antigens bind to the plastic, coating the bottom of the wells.</p>
	<p>The primary antibody is added to the well. In the case of the dengue ELISA, the primary antibodies (IgM) are from the patient's serum sample.</p>
	<p>Excess 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>



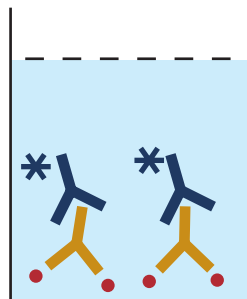
A secondary antibody is added to the wells. This antibody recognizes the patient IgM antibodies, bound to the antigens. The secondary antibody also has a colorimetric tag attached.



Excess secondary antibody is washed away, leaving only secondary antibodies, bound to the patient IgM antibodies. This wash removes excess antibodies that are unbound.



A substrate is added to the wells.



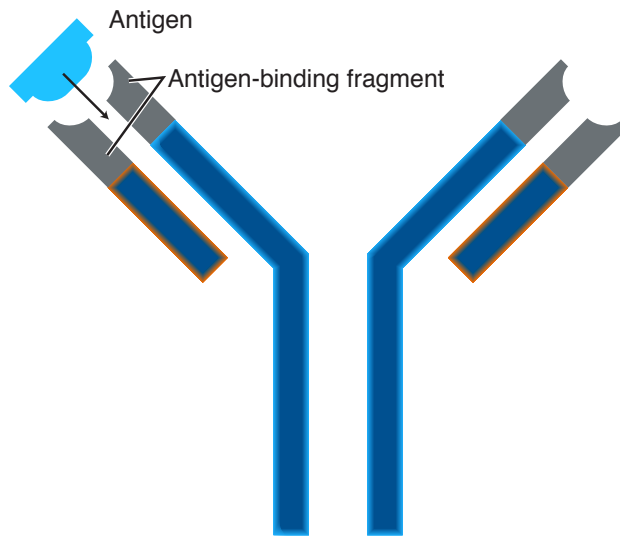
Bound secondary antibody containing a colorimetric tag will cause a color change when exposed to the substrate. A color change indicates a positive reaction.



Antibody



Antigens



Antibody



Testing for Dengue Antibodies

3

Vocabulary:

- Antibody
- Primary antibody
- Antigen
- Secondary antibody
- ELISA

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:

The student will be able to...

1. Perform an ELISA test
2. Explain the use of biotechnology to diagnose disease
3. Recognize that an ELISA is an antibody-based test rather than nucleic acid
4. Explain the steps of an ELISA
5. Propose other uses of an ELISA

Standards:

SC.912.L.16.10 SC.912.L.16.11
SC.912.L.16.12 SC.912.L.18.1

Materials:

If performing the authentic ELISA, this curriculum recommends BioRad. Other companies also have classroom-friendly ELISAs, but the instructions provided here are specific to BioRad.

ELISA test (BioRad's Biotechnology Explorer ELISA Immunoexplorer Kit Catalog #166-2400EDU Protocol III — Antibody test. All necessary consumables are included in the BioRad kit.)

OR

If performing the simulated ELISA, you will need the materials listed below:

- 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

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

TIME ESTIMATE:
• 45 minutes

LEARNING STYLES:
• Visual and kinesthetic

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 research and development.
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:

- Copy Student ELISA Procedure for each student or student pair.
- All directions for performing the ELISA can be found in the instruction manual which accompanies the BioRad kit and are not duplicated here. If using the BioRad ELISA, please follow the preparation instructions included in the kit.
- 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.

Modified ELISA: Simulation Advance Preparation

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 outside 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
Yellow 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	# 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	

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 towels standard in many schools do not adequately absorb liquid, causing samples to splash back and contaminate adjacent wells. This is not 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. For convenience, the continuation of case report 1 is included at the top of the Student ELISA Procedure.

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.

Assessment Suggestions:

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

Modifications:

- Test for different mosquito diseases such as West Nile, Dengue, Yellow Fever
- For advanced classes, teachers may consider extending the lesson to include a quantitative analysis. Instructions for the quantitative analysis are in the BioRad manual.
- 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. (Lesson still in development.)



Student ELISA Procedure

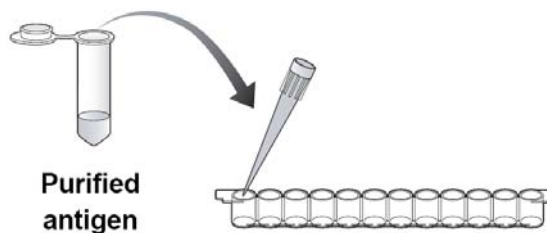
Our story continues.

During the patient's third visit to her physician 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.

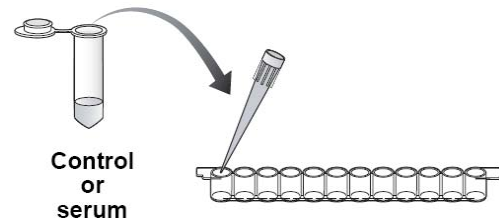
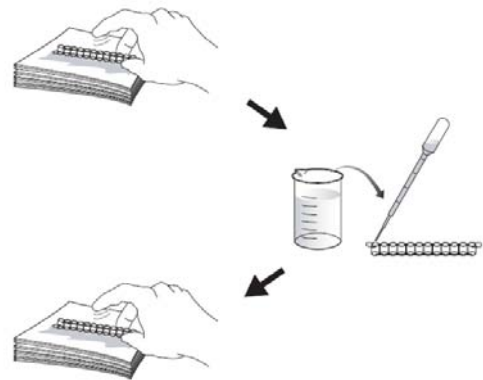
ITEM (LABEL)	CONTENTS	# PER STATION	(☑)
Yellow tube (PAT)	Patient sample (0.25ml)	1	
Violet tube (+)	Positive control (0.5ml)	1	
Blue tube (-)	Negative control (0.5ml)	1	
Green tube (AG)	Purified antigen (1.5ml)	1	
Orange tube (SA)	Secondary antibody (1.5ml)	1	
Brown tube (SUB)	Enzyme substrate (1.5ml)	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.

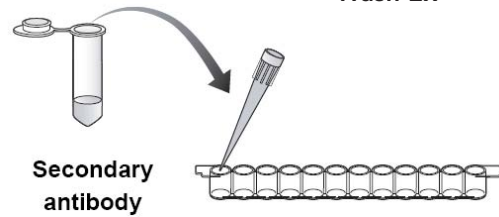




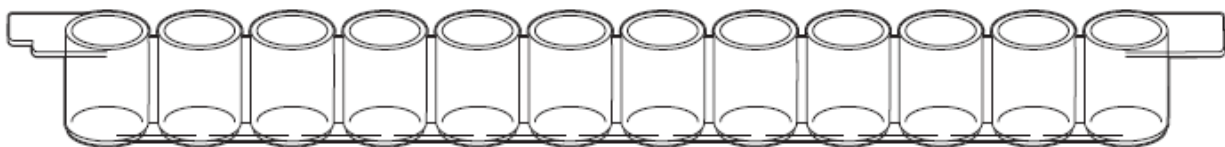
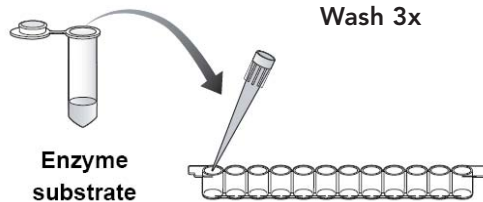
5. Wash:
 - a. Tip the microplate strip upside down onto the paper towels, and tap the strip a few times upside down. Make sure to avoid splashing sample back into wells.
 - b. Discard the top paper towel.
 - c. Use your transfer pipette to fill each well (1-9) with wash buffer, taking care not to spill 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 5
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 5 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 5 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.



Wash 2x



Wash 3x



Gel Electrophoresis

4

Vocabulary:

- Agarose
- Polymerase chain reaction
- cDNA
- RT-PCR
- 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...

1. Explain the process of PCR
2. Describe what the bands on an agarose gel represent
3. Explain how gel electrophoresis works
4. Interpret (and use) the banding pattern on an agarose gel
5. Compare and contrast the use of PCR vs ELISA

Standards:

- SC.912.L.14.52
- SC.912.L.16.11
- SC.912.L.16.12
- SC.912.L.18.1
- SC.912.N.3.5
- SC.912.N.4.1

Materials:

Dyes:

- Xylene cyanol = DENV-1
- Ponceau G= DENV-2
- Bromphenol blue = DENV-3
- Methyl orange= DENV-4

SOURCE	INSTRUCTOR'S (COMMON) WORKSTATION	# REQUIRED
Bio-Rad http://www.bio-rad.com Cat. No. 166-0603EDU, \$299.00	Centrifuge (2.0ml tubes)	1
Fisher Scientific	White light box (optional)	1
Classroom or personal	Digital camera (optional)	1

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

- TIME ESTIMATE:**
- 45 minutes

- LEARNING STYLES:**
- Visual, kinesthetic and auditory

SOURCE	STUDENT WORKSTATION	# REQUIRED PER GROUP	# REQUIRED FOR CLASS
Carolina Biological: Introductory Gel Electrophoresis Teacher Demo Kit Cat # 21-1147 or 8-Station Classroom Kit Cat # 21-1148	PCR samples (bromophenol blue, methyl orange, ponceau G, xylene cyanol)		
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 Methyl orange Ponceau G 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	

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.

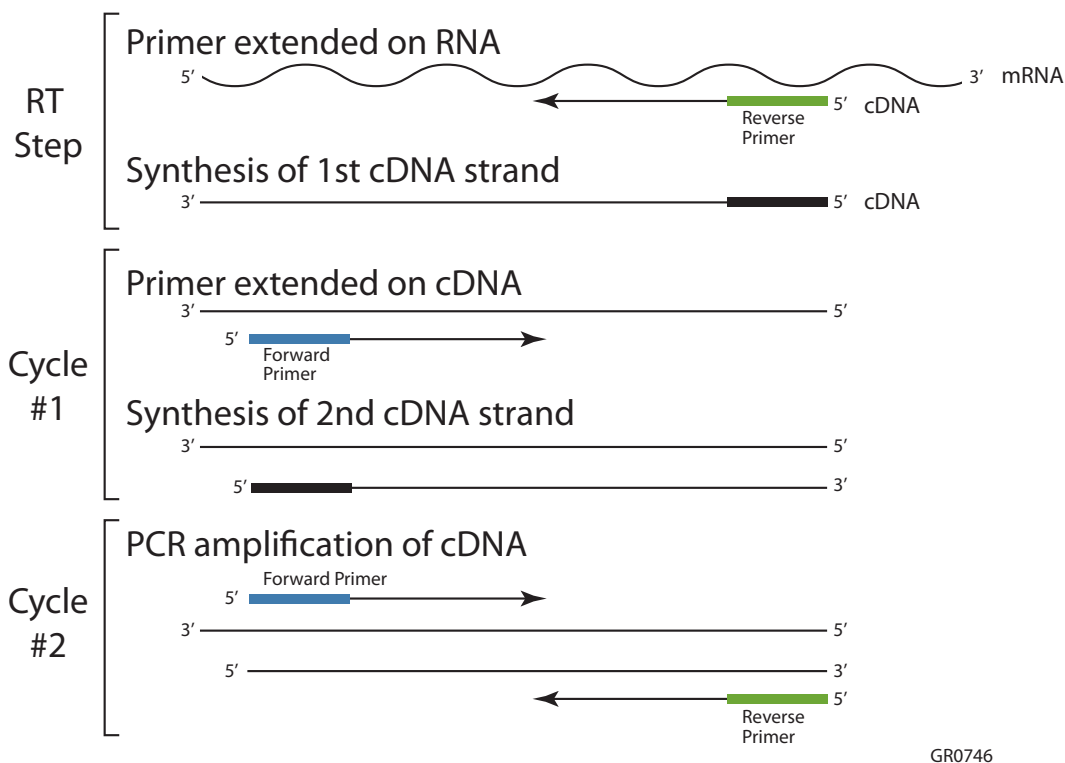
To determine the serotype, a small, unique segment can be amplified using polymerase chain reaction (PCR). Short DNA sequences called primers are designed for each serotype and will only bind and allow amplification of its specific serotype. Dengue virus is an RNA virus. We can still carry out PCR, but 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



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/gelelectrophoresis.html>

Image from Applied Biosystems

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	# 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 is not getting too hot and the samples do not 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. Ask students to recall the ELISA procedure from the previous class and the results. Inform them that since the patient tested positive for Dengue, the CDC and Monroe County Health Department were notified. Since there are four varieties of dengue, 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. Today's task for the students is to determine the patient's serotype.
2. 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.)
3. Distribute Student Worksheet.
4. Allow students time to read the worksheet silently.
5. 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.
6. 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.
7. Instruct students to follow the directions on the worksheet.
8. Remind students to record their results on the epidemiological report.
9. 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 from DNAi: <http://www.dnai.org/teacherguide/>

BioRad's PCR Song: <http://www.cnpg.com/video/flatfiles/539/>

PCR Dash (available to borrow from UF CPET: www.cpet.ufl.edu)

Pipetting activity (available to borrow from UF CPET: www.cpet.ufl.edu)

LITERATURE: Kary Mullis' Nobel Prize Speech: http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1993/mullis-lecture.html

Resources/References:

This protocol is an adaptation of a two-step nested RT-PCR assay described by Lanciotti et al. 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).

Harris, E., Roberts, T. G., Smith, L., Selle, J., Kramer, L. D., Valle, S., ... & Balmaseda, A. (1998). Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. *Journal of Clinical Microbiology*, 36(9), 2634-2639.

Lanciotti, R. S., Calisher, C. H., Gubler, D. J., Chang, G. J., & Vorndam, A. V. (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *Journal of Clinical Microbiology*, 30(3), 545-551.

Pipetting activity is also available at Bokor, J. (2012). You Sank My...Bacteriophage?. *The American Biology Teacher*, 74(6), 422-423.

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://learn.genetics.utah.edu/content/labs/pcr/(virtual%20lab))

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



Gel Electrophoresis

The Case Continues

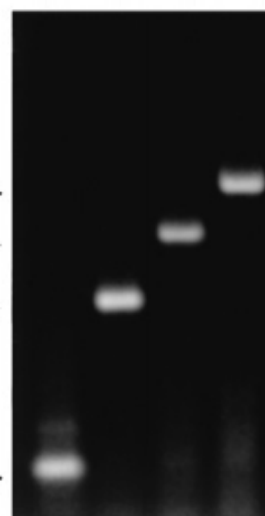
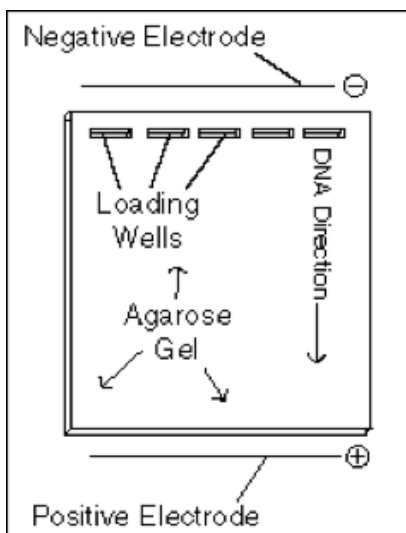
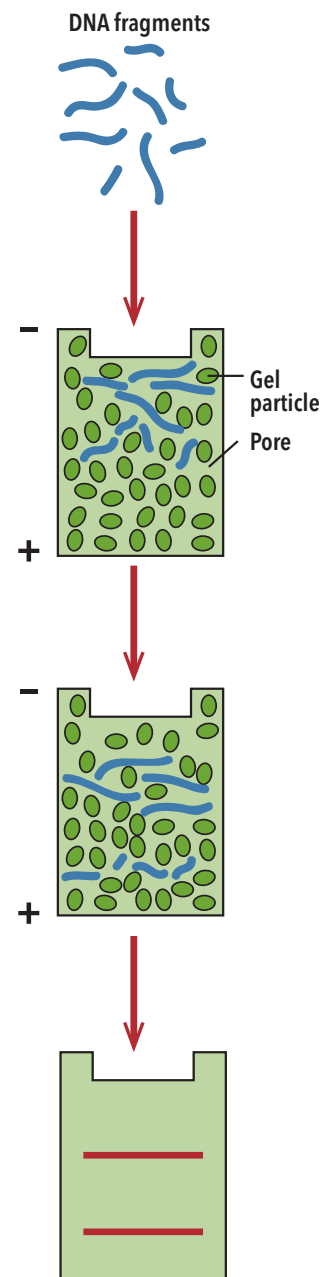
Testing of a serum specimen at a private laboratory revealed dengue immunoglobulin M (IgM) antibodies. After her physician notified Monroe County Health Department (MCHD) of the test result, the patient's serum specimen from August 17, a cerebral spinal fluid (CSF) specimen from August 13, and a repeat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



DNA Gel Electrophoresis Lab

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?

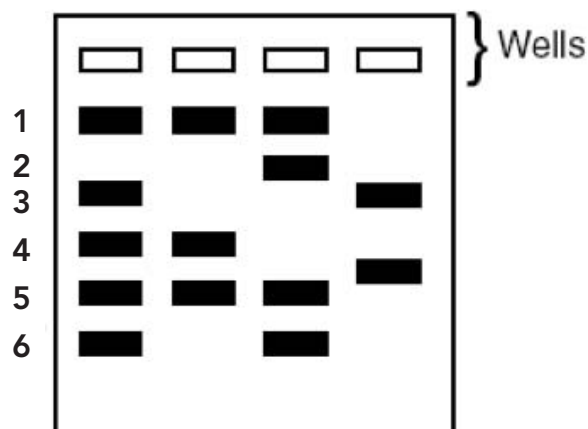
2. Describe how different sized DNA fragments are separated by the agarose gel matrix.

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

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?

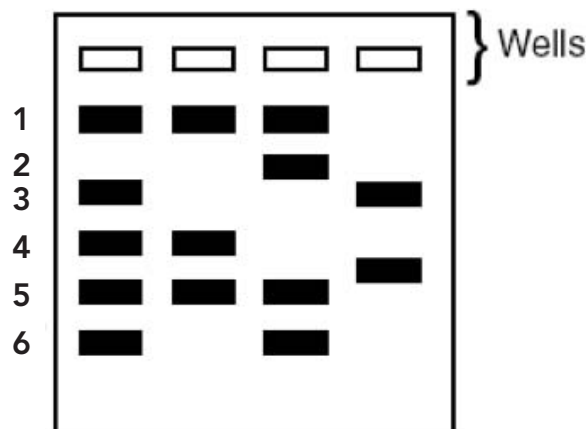
1

- 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.



Different Tests for Different Stages

5

Vocabulary:

- 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...

1. Compare and contrast ELISA and RT-PCR
2. Explain the difference between antibodies (proteins) and DNA and know which test uses each macromolecule
3. Interpret a graphical model of immune response
4. Identify that viral load is highest at the on-set of an illness and decreases as the body's immune system responds to the infection

Standards:

SC.912.L.14.52 SC.912.L.16.10 SC.912.L.16.11
SC.912.L.18.1 SC.912.N.1.6 SC.912.N.4.2

Materials:

Student worksheet (one per student)

Background Information:

As with most infections, the titers of the infectious agent are usually highest at the onset 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 dengue virus infection, but antibody enhancement can actually cause a more severe secondary reaction, leading to dengue hemorrhagic fever.

? KEY QUESTION(S):

- Why are different molecular tests used to diagnose dengue virus?

🕒 TIME ESTIMATE:

- 30 minutes

🧠 LEARNING STYLES:

- Visual

Teaching tip: 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, the NS1 ELISA 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.
8. Either before class ends or the following period, discuss the answers to the student worksheet to ensure understanding of all students.
9. Present the students with the case closed story wrap by either providing copies for them to read or read aloud.
10. Invite discussion of the measures taken by Monroe County and future episodes.

Assessment Suggestions:

Worksheet can be collected.

Modifications:

For advanced students, print out or direct students to Host Response to the Dengue Virus (link below). 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:

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>

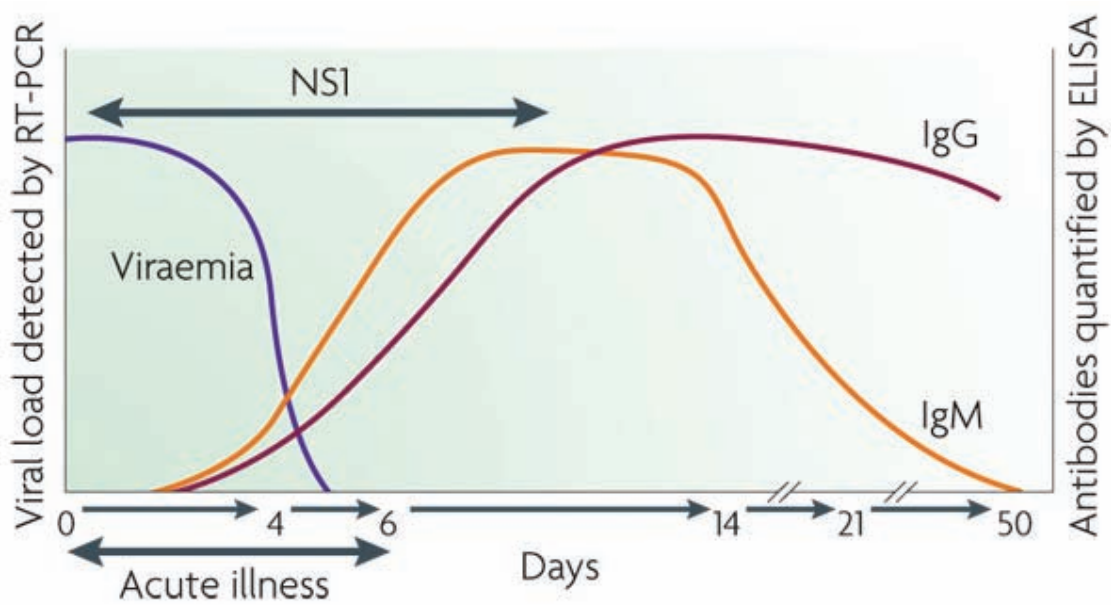
Resources/References:

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

Guzman, M. G. et al. Dengue: A continuing global threat. *Nature Reviews Microbiology* 8, S7–S16 (2010). doi:10.1038/nrmicro2460



Answer the following questions using the graph below.

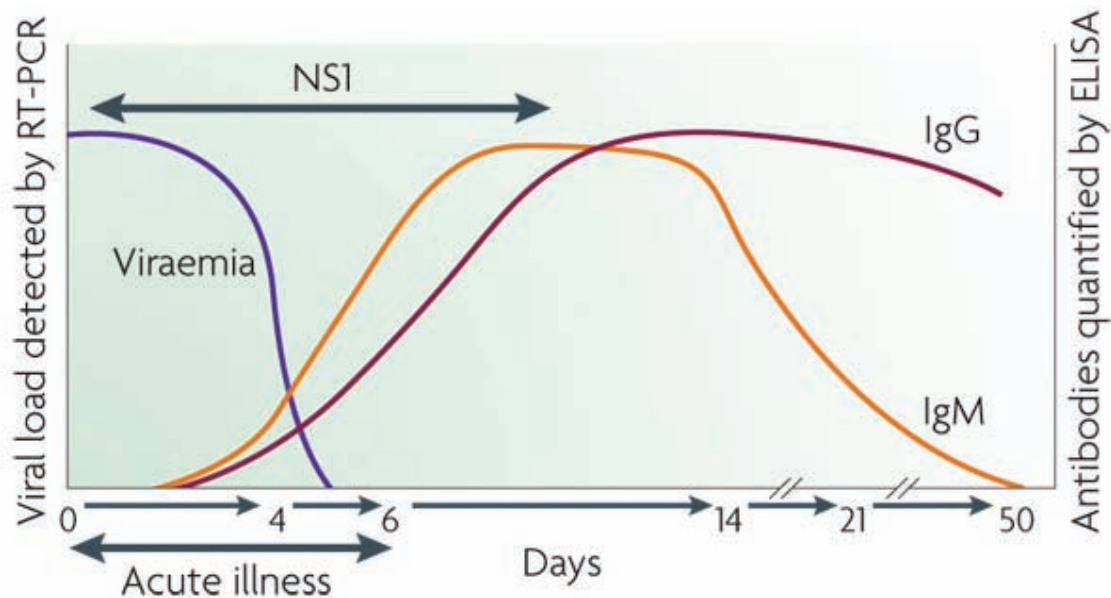


<http://www.nature.com/scitable/topicpage/host-response-to-the-dengue-virus-22402106>

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?



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?

~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.

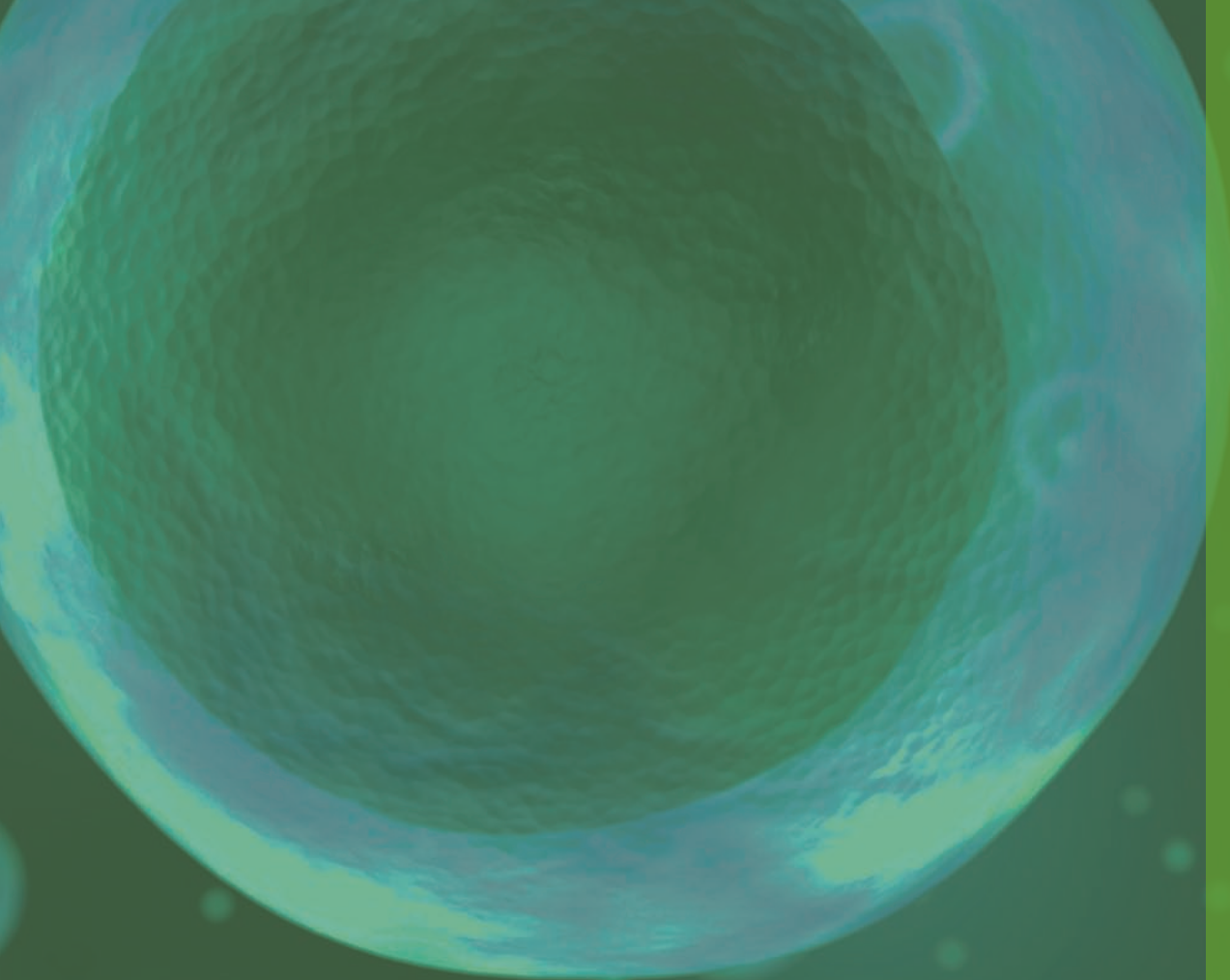


Case Closed

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 Monroe County Health Department (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, 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. 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.



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