Different Tests for Different Stages

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.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.
**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://peertamu.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
Answer the following questions using the graph below.

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?

http://www.nature.com/scitable/topicpage/host-response-to-the-dengue-virus-22402106
Answer the following questions using the graph below.

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 <i>Aedes aegypti</i> 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.