Testing for Dengue Antibodies

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.

<table>
<thead>
<tr>
<th>TUBES (NUMBER NEEDED)</th>
<th>DESCRIPTION</th>
<th>LABEL</th>
<th>CONTENTS (EACH TUBE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violet tubes, 8</td>
<td>Positive controls</td>
<td>+</td>
<td>0.5ml water</td>
</tr>
<tr>
<td>Blue tubes, 8</td>
<td>Negative controls</td>
<td>–</td>
<td>0.5ml water</td>
</tr>
<tr>
<td>Green tubes, 8</td>
<td>Purified antigen</td>
<td>AG</td>
<td>1.5ml water</td>
</tr>
<tr>
<td>Orange tubes, 8</td>
<td>Secondary antibody</td>
<td>SA</td>
<td>1.5ml water</td>
</tr>
<tr>
<td>Brown tubes, 8</td>
<td>Enzyme substrate</td>
<td>SUB</td>
<td>1.5ml water</td>
</tr>
<tr>
<td>Yellow tubes, 8</td>
<td>Patient sample</td>
<td>PAT</td>
<td>0.25ml water</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>ITEM (LABEL)</th>
<th>CONTENTS</th>
<th># PER STATION</th>
<th>(E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow tube (PAT)</td>
<td>Patient sample (0.25ml)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Violet tube (+)</td>
<td>Positive control (0.5ml)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Blue tube (–)</td>
<td>Negative control (0.5ml)</td>
<td>1</td>
<td></td>
</tr>
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<td>Purified antigen (1.5ml)</td>
<td>1</td>
<td></td>
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<td>Secondary antibody (1.5ml)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Brown tube (SUB)</td>
<td>Enzyme substrate (1.5ml)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Beaker of wash buffer</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>12-well microplate strip</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Disposable transfer pipette</td>
<td></td>
<td>7 (only 1 needed for wash buffer if using P200)</td>
<td></td>
</tr>
<tr>
<td>20-200ul micropipette</td>
<td></td>
<td>1 (if available)</td>
<td></td>
</tr>
<tr>
<td>20-200ul tips</td>
<td></td>
<td>1 box (if available)</td>
<td></td>
</tr>
<tr>
<td>Stack of paper towels</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
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.

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</tr>
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</table>

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.

Images from BioRad