Steps of an ELISA

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

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

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a “sandwich” ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.
Advance Preparation:

- 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.

Assessment Suggestions:
Instructor can visually observe correct completion of the activity.

Resources/References:
ELISA video: [http://www.youtube.com/watch?v=RRbuz3VQ100&feature=related](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.

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

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<thead>
<tr>
<th>Steps</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Virus proteins" /></td>
<td>Virus proteins (antigens) are added to wells of a 96-well plate.</td>
</tr>
<tr>
<td><img src="image" alt="Antigens bind" /></td>
<td>The antigens bind to the plastic, coating the bottom of the wells.</td>
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Antigens

Antigen-binding fragment

Antibody