Understanding and Interpreting the ELISA

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Abstract

This activity is designed to provide a visual demonstration of how the indirect enzyme-linked immunosorbent assay (ELISA) works. Four students play roles as either technician, washer, patient "A", or patient "B". Sponges are cut into appropriate shapes to represent various antigens and Y-shaped antibody molecules which contain variable regions complementary in shape to the antigens. Sponges are also used to represent the enzyme-labeled secondary antibody. As the drama unfolds, students should be able to visualize what is happening at the molecular level in the various wells of an ELISA plate (positive control, negative control, and experimental wells). It can also help students understand the ELISA in an educational setting where it cannot be carried out as a lab exercise because the necessary equipment or funds are not available.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Time Required. 20 minutes

Pedagogical Function.
Learning objective. Students will be able to describe the indirect ELISA technique and accurately explain why the results of a test are positive or negative.
Learning outcome. Students will be able to describe the indirect ELISA technique, identify the function of the various reagents, and accurately interpret both negative and positive results.
Approach. The activity may be performed in groups or as a class in either lecture or the laboratory. Although helpful, no homework or research is required for the simulation. However, homework is required to correctly answer some of the assessment questions at the end of the activity.

Background.
Students need no prior knowledge if the instructor wishes to direct this activity. However, some of the questions in the assessment portion of this activity do require background information. If the instructor wishes students to direct and explain the activity themselves, students should understand the concepts of antigen, antibody, variable region (antigen-binding site), constant region, enzyme, and substrate.

PROCEDURE

Materials.
Large, clear glass or plastic bowls - 4

Paper or carboard for placards - 4
Marker for writing on placards and tape labels
Sponges - 18 (large, soft sponges often used for washing automobiles are recommended but others will work)
Scissors or electric carving knife to cut sponges
Tape
Velcro - 2 yards
Glue (for attaching Velcro to sponges)
Straws - 4
Plastic bags (e.g., Ziploc) - 7
Wash bottle
Cellophane, 20- by 20-cm clear pieces - 4
Cellophane, 20- by 20-cm blue pieces - 4

Instructor Version.

A. Antigens in wells. Obtain four large clear glass or plastic bowls and six large clean sponges. Use tape to label the bowls as "Well 1", "Well 2", "Well 3", and "Well 4". Each sponge represents an antigen. Four of the sponges are cut so as to be identical to one another. Prepare the four sponges by cutting away portions of the outer edge so that the sponges resemble an interlocking puzzle piece with triangle-shaped projections of the same size and shape on each sponge. These protruding projections represent epitopes. An example is shown in Fig. 1. The ELISA is designed to detect specific antibodies that will bind to these four antigens (sponges).

FIG. 1. Sponge cut to represent an antigen with protruding epitopes.

The other two sponges represent antigens that are different from the first four. Prepare these two sponges by cutting away portions of the outer edge that are unique (different from the shape of the four sponges and from each other). These epitopes are invaginated. Examples are shown in Fig. 2.

FIG. 2. Two sponges cut to represent antigens with invaginated epitopes.

Tape one of the identically-shaped sponges into each well. Make sure that at least one side that has epitopes on it is projecting upward in each bowl (Fig. 3).

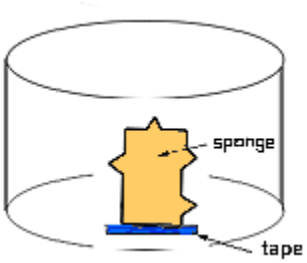

FIG. 3. Well with a sponge taped into it so that at least one epitope points upward.

Also tape one of the nonidentical sponges into Well 3 and another nonidentical sponge into Well 4. Make sure that at least one side that has epitopes on it is projecting upward in each bowl (Fig. 4).

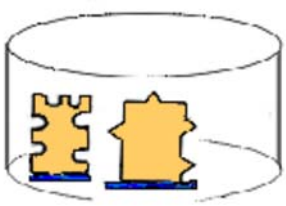

FIG. 4. Well with one of the identical sponges and one of the nonidentical sponges taped into it so at least one of their

epitopes points upward.

Glue Velcro around each epitope shape on all six sponges. These are the "antigens".

B. Positive control packet. Cut a sponge into a Y-shape so it resembles an immunoglobulin G (IgG) antibody molecule. In the section of the sponge representing the variable (antigen-binding) region, cut out a complementary shape to fit the epitope of the antigen shown in Fig. 1. Glue the corresponding piece of Velcro on the variable region so that it will bind to the Velcro on the epitope of the antigen shown in Fig. 1. Also glue Velcro onto the Fc region of the antibody molecule (Fig. 5). Place in a plastic bag labeled "Positive Control".

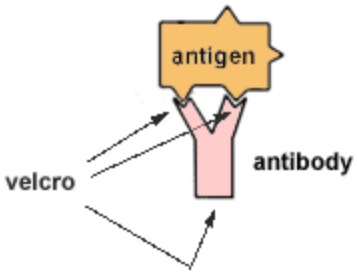

FIG. 5. Sponge cut to resemble an IgG antibody molecule complementary to the antigen in Fig.1 with velcro attached to the variable and Fc regions.

C. Negative control packet. Cut a sponge into a Y-shape so it resembles an IgG antibody molecule. In the section of the sponge representing the variable region, cut out shapes that are not complementary to the epitopes shown in Fig. 1 (Fig. 6). Glue the corresponding piece of Velcro on this variable region as indicated in part B. Place in a plastic bag labeled "Negative Control".

FIG. 6. Sponge cut to resemble an IgG antibody molecule whose variable region is not complementary to the antigen in Fig. 1.

D. Patient "A" packet. Prepare a placard with the words "Patient A" that a student can wear (e.g., attached to clothing with a safety pin or using a string to hang the placard around the neck). Cut a sponge into a Y-shape so it resembles an IgG antibody molecule with variable regions complementary for the antigen shown in Fig. 1. In addition, cut two sponges into Y-shaped IgG molecules whose variable regions have a shape that is not complementary to the epitopes of the antigen shown in Fig. 1. Glue Velcro to the sites as noted for the positive-control antibody in part B. Place in a plastic bag labeled "Patient A".

E. Patient "B" packet. Prepare a placard with the words "Patient B" that a student can wear. Cut three sponges into a Y-shape so they resemble IgG antibody molecules with variable regions noncomplementary for the antigen shown in Fig. 1. Glue the corresponding piece of Velcro on this variable region as in part B and glue Velcro on the Fc region of each antibody molecule. Place in a plastic bag labeled "Patient B".

F. Washer packet. Prepare a placard with the word "Washer" that a student can wear. Include an empty squirt bottle to represent the wash buffer. Place in a plastic bag labeled "Washer".

G. Technician packet. Prepare a placard with the word "Technician" that a student can wear.

H. Substrate. Prepare four 20- by 20-cm pieces of clear cellophane which represent the enzyme substrate. Also prepare four pieces of blue cellophane of the same size which represent the color change that occurs during enzyme-substrate interaction. Place in a plastic bag labeled "Substrate".

I. Enyzme-labeled secondary antibody packet. Prepare four Y-shaped IgG antibodies made of sponges as above, but cut shapes in the variable regions so they will interact with the Fc region of the Positive Control and Patient "A" antibodies. Glue appropriately placed Velcro on to these secondary antibodies (in the variable, antigen-binding regions). Insert straws into the Fc region of this sponge to represent bound enzyme. Straws may be glued into place or attached using Velcro (Fig. 7). Place in a plastic bag labeled "Secondary Antibody".

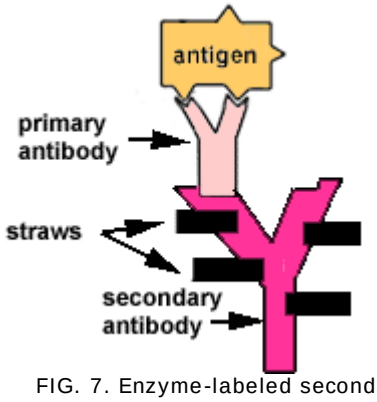

FIG. 7. Enzyme-labeled secondary antibody.

J. Four student volunteers. Solicit four students from the class to participate in the demonstration.

Student Version.

Positive control.

a. Student wearing the "Technician" placard places the Positive Control antibody (sponge) into Well 1 with Velcro used to bind to epitopes on the antigen. The shape of the anitbody and the epitope are complementary to one another. Explain that the "rules of this simulation" are that antibodies are considered to be bound only if the Velcro on the antibody attaches at all points to the Velcro on an epitope. If the Velcro doesn't attach at all points (if it is not complementary), the antibody is not binding and will wash out.
b. Student wearing "Washer" placard pretends to wash the antibody and antigen with the squirt bottle. Since all antibody is bound (complementary), none will wash away. If there was unbound antibody (noncomplementary) in this well, it would wash out.
c. "Technician" adds enzyme-labeled Secondary Antibody which binds via Velcro to the constant region of the attached positive antibody.
d. "Washer" repeats step b. Any unbound antibodies would be washed away. In this case, all antibodies are bound and do not wash out.
e. "Technician" places the Substrate (clear cellophane) over the bound enzyme-labeled Secondary Antibody in the well, then places blue cellophane over the clear to represent the color change that occurs after the enzyme binds the substrate (Fig. 8).

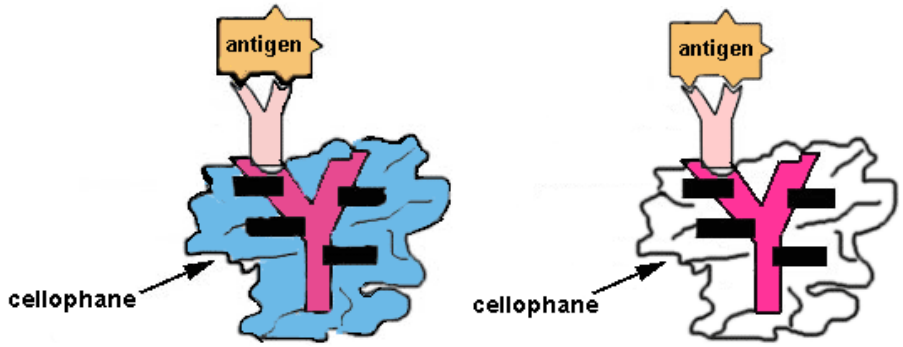

FIG. 8. Clear cellophane (representing substrate) and blue cellophane (representing the color change that occurs after the enzyme binds the substrate) over the bound enzyme-labeled secondary antibody.

Negative control.

f. "Technician" places Negative Control antibody (sponge) in Well 2. The shapes of the antibody and the epitopes are not complementary to one another. Explain that for modeling purposes, we have chosen to make the epitopes on the complementary antigens protruding and the epitopes on the noncomplementary antigens invaginated. This is to prevent any random sticking activity. In reality, complementary and noncomplementary antigens may have protruding epitopes of varying shapes.
g. Student wearing "Washer" placard pretends to wash the antibody and antigen with the squirt bottle. Since all antibody is unbound (noncomplementary), it will wash away (out of the well).
h. "Technician" adds enzyme-labeled Secondary Antibody.
i. "Washer" repeats step g. All antibodies are unbound and wash out.
j. "Technician" places the Substrate (clear cellophane) over the antigen in the well. No blue cellophane is added because the enzyme and substrate did not bind.

Patients.

k. "Patient A" places all three of his/her antibodies (one complementary to the antigen, two noncomplementary) into Well 3. The procedure is repeated as described above with the positive and negative controls. The complementary antibody will bind to the antigen it is specific for and remain in the well. The noncomplementary antibodies (complementary to some other types of antigens) will wash out. Blue cellophane will be added over the clear cellophane to represent the color change that occurs after the enzyme acts upon the substrate.

l. "Patient B" places all three of his/her noncomplementary antibodies into Well 4. The procedure is repeated as described above with the negative control. The noncomplementary antibodies will wash out. No blue cellophane is added because the substrate and enzyme did not bind.

Safety Issues.
No safety issues are involved in this activity.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
Student outcomes may be assessed by posing the questions listed below either in class or as take-home assignments. If class time is used, students may be divided into groups and use demonstration materials to illustrate their answers.

a. Explain why a positive ELISA reaction occurs. Explain what the colors indicate (clear versus blue). [comprehension]
b. Illustrate how a false positive or false negative reaction could occur. [comprehension]
c. Explain why rinsing the well thoroughly is so important. [comprehension]
d. Demonstrate how to test the enzyme stability of the secondary enzyme-labeled antibody in the case of an unexpected negative reaction, for example, if no color change is seen in your positive control. [application]
e. Propose what secondary antibody you would use to see if the results of the ELISA were due to a primary or secondary exposure. [synthesis]
f. Evaluate how you could determine the titer of this antibody. [evaluate]
g. If you were testing for the presence of an antigen (such as a hormone or a cytokine) in a patient's serum, how would you modify the ELISA? [synthesis]
h. Demonstrate or dramatize what happens with inadequate washing in a negative control. [application]
i. Compare and contrast or dramatize a direct and indirect sandwich ELISA. [analysis/application]
j. Appraise the flexibility of the ELISA which allows it to be used for diagnosing a variety of diseases. [evaluate]
k. Organize the steps involved in performing an ELISA for HIV testing. [synthesis]
l. List the role for each reagent involved in the ELISA. [comprehension]
m. Generate a concept map for the ELISA procedure, specifically for HIV testing. [synthesis]