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AUTHOR Baker, William P.; Moore, Cathy R.
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ABSTRACT

Understanding of immunological techniques such as the Enzyme Linked Immuno Sorbent Assay (ELISA) is an important part of instructional units in human health, developmental biology, microbiology, and biotechnology. This paper describes a simple ELISA exercise for undergraduate biology that effectively simulates the technique using a paper model. This hands-on procedure is designed for use as either a laboratory or classroom exercise. (JRH)

A Simple ELISA Exercise for Undergraduate Biology

William P. Baker and Cathy R. Moore
College of Science and Allied Health
Grand Canyon University
Phoenix, Arizona 85017

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Understanding of immunological techniques such as the Enzyme Linked Immuno Sorbent Assay (ELISA) is an important part of instructional units in human health, developmental biology, Microbiology and biotechnology. Experience, however, indicates that the topic is a difficult one for many students. We have developed a simple ELISA exercise for undergraduate Biology that effectively simulates the technique using a paper model. The hands-on procedure outlined below is designed for use as either a laboratory or classroom exercise. Instructors may use the templates provided or create their own. Adapting manipulatives to fit topics being presented enhances instruction and student satisfaction.

Time Required:

1 laboratory or class period

Materials:

1 copy of Figure 1 for each student
1 copy of Figure 2 per group on colored paper
1 copy of Figure 3 for each student
Scissors
Tape

Objectives:

To explain the ELISA technique.
To explain how the reaction becomes visible to the unaided eye.

Procedure:

Students are told that enzymes function as catalysts and act on substrates to produce many types of products. To be useful in a laboratory as a diagnostic tool, enzymes are chosen that cause fluorescent, luminescent, or colorful changes in the substrates. These changes can be detected by the unaided eye or using special instrumentation. An example of a diagnostic enzyme immunoassay that can be detected with the unaided eye is the Enzyme Linked Immuno Sorbent Assay. This test is usually referred to as ELISA.

The ELISA method is then modeled using a simple physical analogue and hands-on activity. Students work individually or in small groups (see Table 1). Each student is instructed to begin by selecting an antibody sequence from a copied sheet (see Figure 1). This figure represents an antibody attached to well of the ELISA assay dish. By analogy with

antigen binding to this attached antibody, the student then cuts and puts together an antigen (see Figure 2) that is complementary to the binding site of the immobilized, primary antibody.

Students are next instructed to cut and arrange a second antibody complementary to a different site on the immobilized antibody-antigen complex (see Figure 3). As with ELISA, the second antibody is shown covalently linked to an enzyme (such as alkaline phosphatase). This enzyme is capable of rapidly converting a colorless substrate into a visible colored product. Students see that the amplification of this reaction makes a positive test visible to the unaided eye when the instructor places a brightly colored sticker (not included) at the point of catalytic action. The finished substrate-enzyme complex tagged with the visible enzyme complex should look like Figure 4.

TABLE 1. Rules for Creating Antibodies.

1. Work individually or in groups of two.

2. Each person choose one immobilized antibody sequence to cut out.

3. Cut out your selection and tape together an antigen complementary to the binding site of the immobilized antibody.

4. Cut out and arrange a second antibody complementary to a different site on the immobilized antibody-antigen complex (The second antibody must be linked to an enzyme).

5. Your instructor will supply you with a brightly colored sticker to demonstrate the catalytic action of this enzyme.

Conclusion:

Research indicates that the effectiveness of instruction is enhanced when it incorporates materials that actively engage students in the generation of scientific explanations. To this end, the present exercise allows students to model the ELISA technique using readily available resources. Students' comments indicate this hands-on experience to be beneficial. As one student responded when asked on a survey to comment about this laboratory exercise, "the opportunity to work hands-on with the ELISA model provided me with the visualization I needed to fully understand."

Sample Questions:

1. Diagram and explain the ELISA technique.

2. Explain how the reaction becomes visible to the naked eye.

3. Define the following:

    a. substrate

    b. antigen

4. Describe specific differences between the real antibodies used in ELISA and the cut-out figures you used.

5. Explain how a positive ELISA test can be visible to the unaided eye.

References:

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Nester, E.; Roberts, C.E.; Nester, M. (1995). Microbiology:a human perspective. Dubuque: W. C. Brown.

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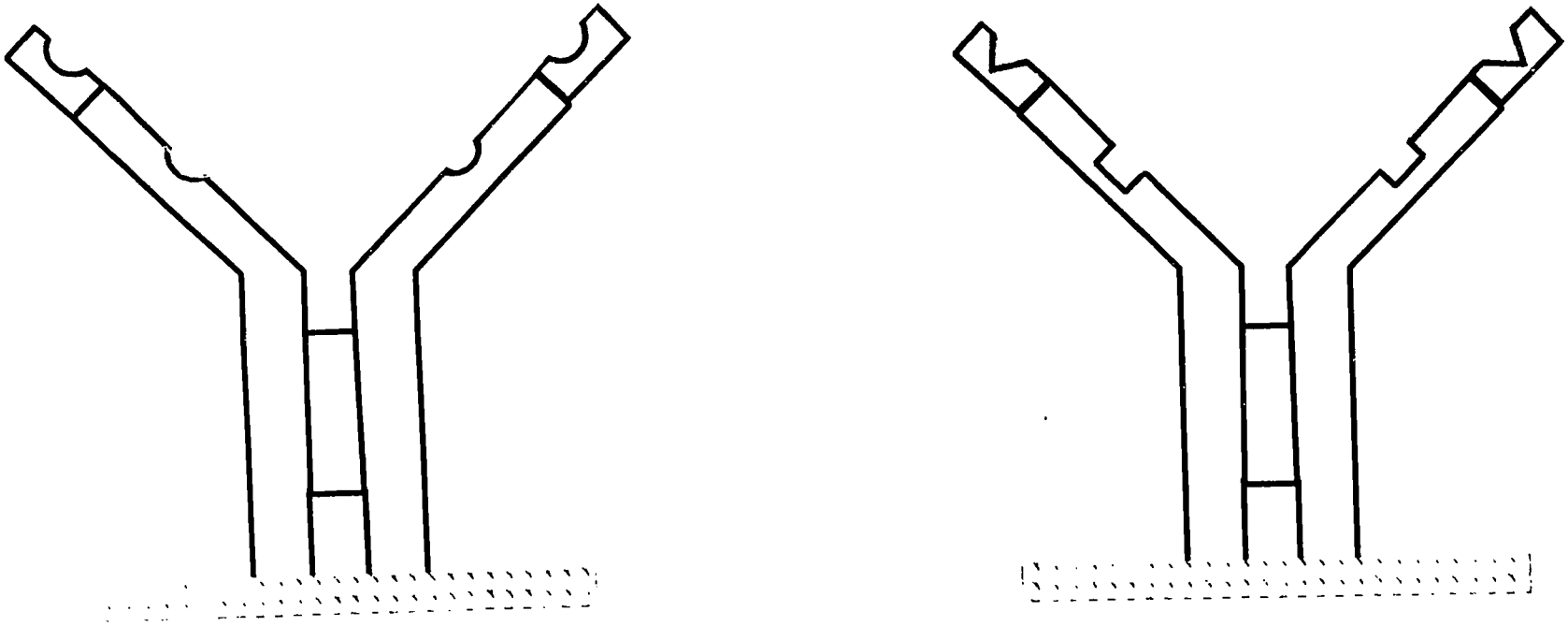

Figure 1: Immobilized antibody

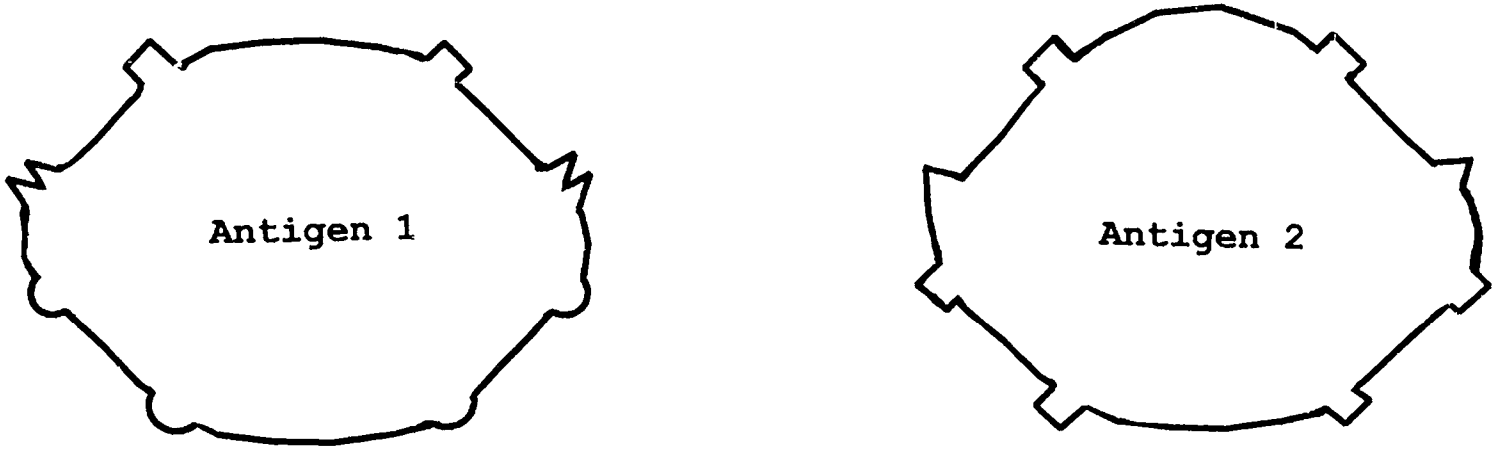

Figure 2: Antigens

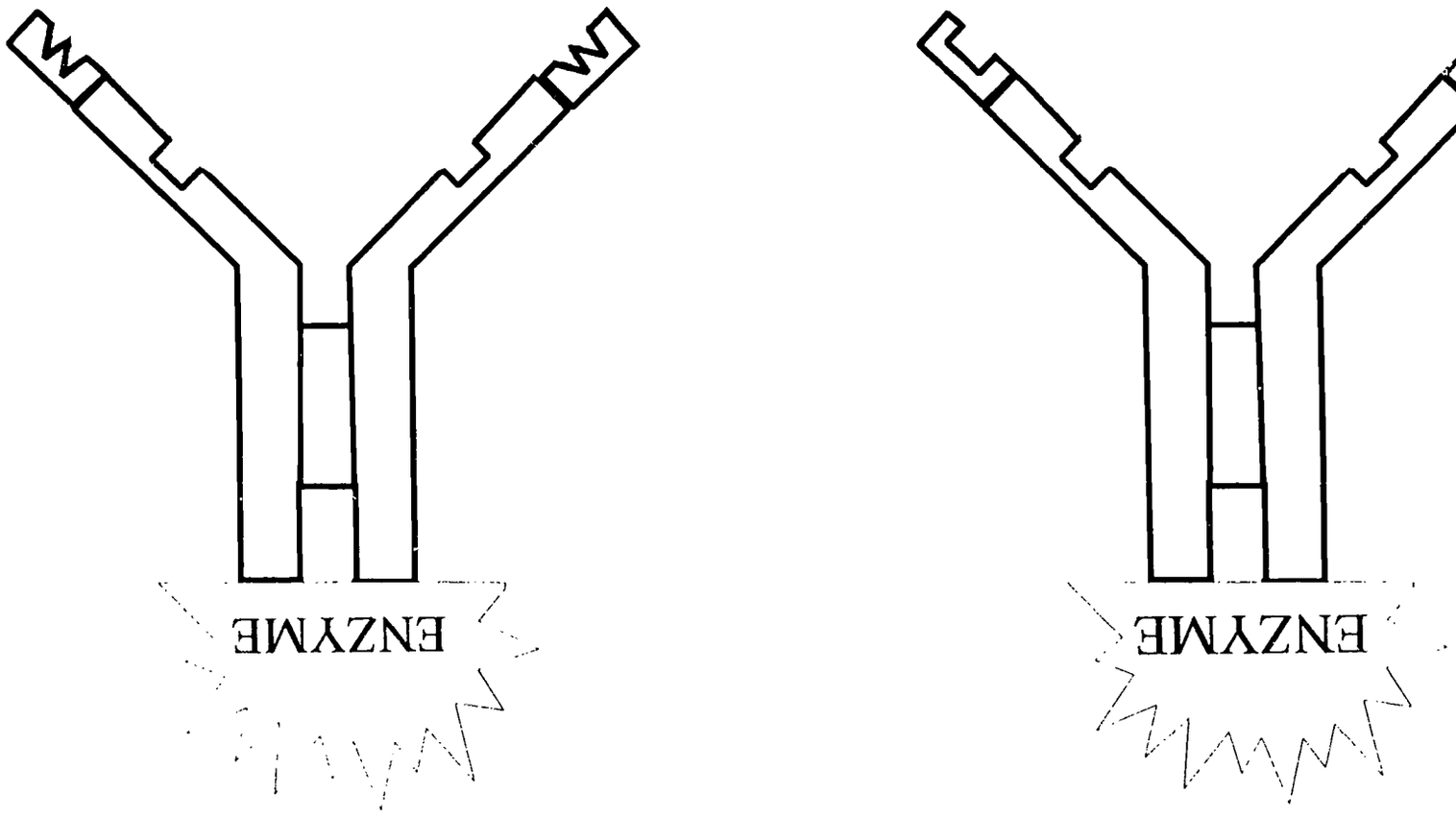

Figure 3: Antibody covalently linked to enzyme

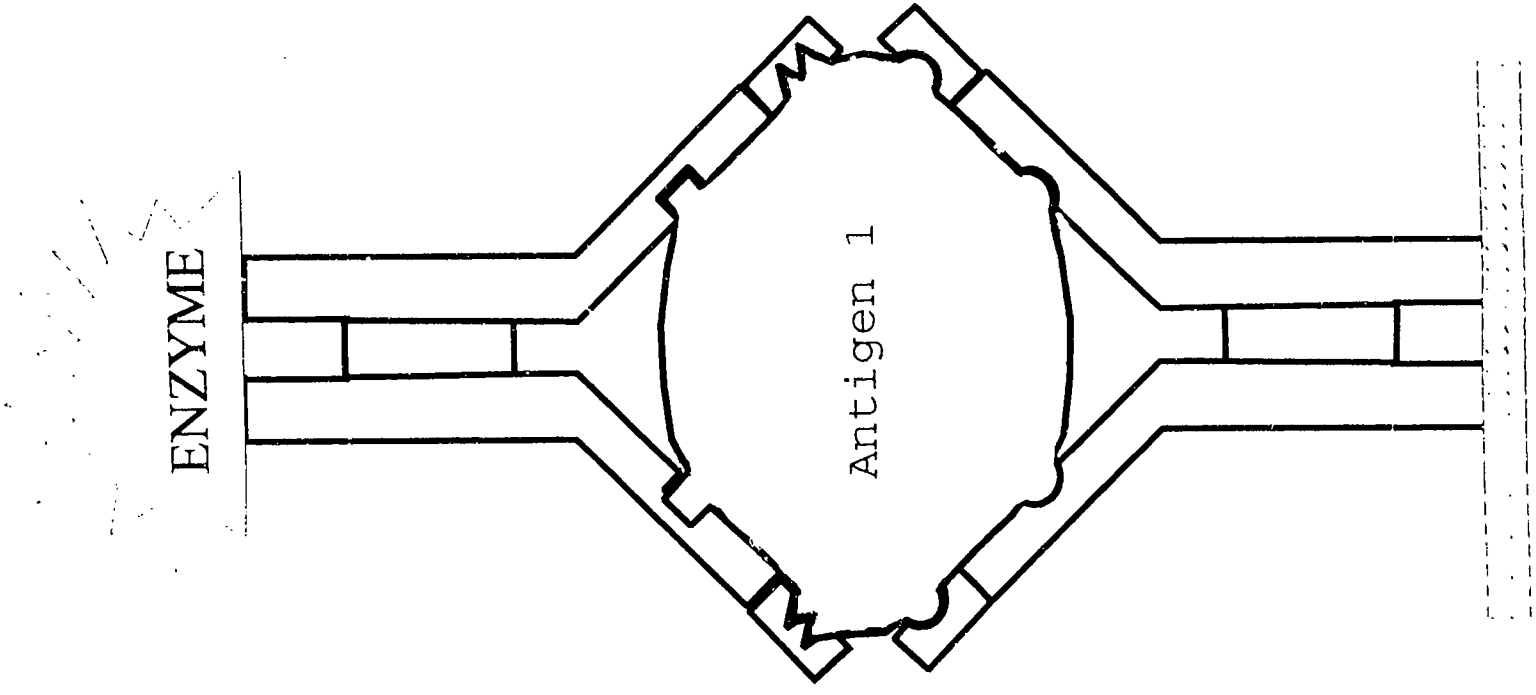

Figure 4: Diagram of finished enzyme-substrate complex