Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay

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INTRODUCTION

Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are both widely used as diagnostic tools in medicine and as quality control measures in various industries; they are also used as analytical tools in biomedical research for the detection and quantification of specific antigens or antibodies in a given sample. These two procedures share similar basic principles and are derived from the radioimmunoassay (RIA). RIA was first described by Berson and Yalow (Yalow and Berson, 1960), for which Yalow was awarded the Nobel Prize in 1977, to measure endogenous plasma insulin. RIA was then developed into a novel technique to detect and measure biological molecules present in very small quantities, paving the way for the analysis and detection of countless other biological molecules, including hormones, peptides, and proteins. Because of the safety concern regarding its use of radioactivity, RIA assays were modified by replacing the radioisotope with an enzyme, thus creating the modern-day EIA and ELISA.

GENERAL PRINCIPLES

EIA/ELISA uses the basic immunology concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample. EIA and ELISA utilize enzyme-labeled antigens and antibodies to detect the biological molecules, the most commonly used enzymes being alkaline phosphatase (EC 3.1.3.1) and glucose oxidase (E.C. 1.1.3.4). The antigen in fluid phase is immobilized, usually in 96-well microtiter plates. The antigen is allowed to bind to a specific antibody, which is itself subsequently detected by a secondary, enzyme-coupled antibody. A chromogenic substrate for the enzyme yields a visible color change or fluorescence, indicating the presence of antigen. Quantitative or qualitative measures can be assessed based on such colorimetric reading. Fluorogenic substrates have higher sensitivity and can accurately measure levels of antigen concentrations in the sample. The general procedure for ELISA is outlined in Figure 1.

Various types of ELISAs have been employed with modification to the basic steps described in Figure 1. The key step in the ELISA assay is the direct or indirect detection of antigen by adhering or immobilizing the antigen or antigen-specific capture antibody, respectively, directly onto the well surface. For sensitive and robust measurements, the antigen can be specifically selected out from a sample of mixed antigens via a

WHAT ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) DOES

- ELISA is a biochemical assay that uses antibodies and an enzyme-mediated color change to detect the presence of either antigen (proteins, peptides, hormones, etc.) or antibody in a given sample.
- Both "indirect" and "sandwich" ELISAs allow detection of antigen or antibody at very low concentrations.
- The competitive method detects compositional differences in complex antigen mixtures with high sensitivity, even when the specific detecting antibody is present in relatively small amounts.
- Multiple and portable ELISA is a ready-to-use, low-cost lab kit that is ideal for large population screening in low-resource settings.

LIMITATIONS

- The enzyme-mediated color change will react indefinitely. Over a sufficiently long period of time, the color strength will inaccurately reflect the amount of primary antibody present, yielding false-positive results.
- To detect a given antibody or antigen, a known reciprocal antigen or antibody must be generated.
- Nonspecific binding of the antibody or antigen to the plate will lead to a falsely high-positive result.

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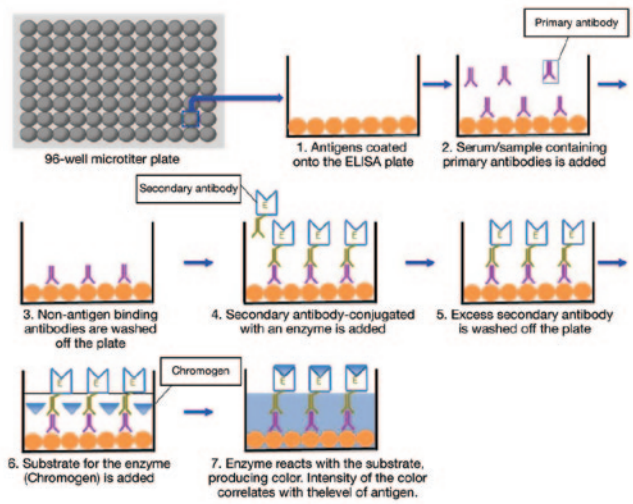

Figure 1. Enzyme-linked immunosorbent assay (ELISA) technique used to detect an antigen in a given sample. The antigen (in liquid phase) is added to the wells, where it adheres to the walls. Primary antibody binds specifically to the antigen. An enzyme-linked secondary antibody is added that reacts with a chromogen, producing a color change to quantitatively or qualitatively detect the antigen.

"capture" antibody. The antigen is thus "sandwiched" between such capture antibody and a detection antibody. If the antigen to be measured is small in size or has only one epitope for antibody binding, a competitive method is used in which either the antigen is labeled and competes for the unlabeled antigen–antibody complex formation or the antibody is labeled and competes for the bound antigen and antigen in the sample. Each of these modified techniques of ELISA can be used for a qualitative and quantitative purpose.

TYPES OF ELISA

Indirect ELISA

A sample that must be analyzed for a specific antigen is adhered to the wells of a microtiter plate, followed by a solution of nonreacting protein such as bovine serum albumin to block any areas of the wells not coated with the antigen. The primary antibody, which binds specifically to the antigen, is then added, followed by an enzyme-conjugated secondary antibody. A substrate for the enzyme is introduced to quantify the primary antibody through a color change. The concentration of primary antibody present in the serum directly correlates with the intensity of the color. One application of the indirect ELISA method is demonstrated by Haapakoski *et al.* (2013), who investigated the role of Toll-like receptor activation during cutaneous allergen sensitization using ovalbumin (OVA) in the modulation of allergic asthma. In one experiment, dermal exposure to Toll-like receptor ligands (lipopolysaccharide, Pam_3Cys, P(I:C)) was demonstrated to downregulate OVA-specific IgE antibodies in serum, as measured by the indirect ELISA technique (Figure 2).

A main disadvantage of indirect ELISA is that the method of antigen immobilization is not specific. When serum is used as the test antigen, all proteins in the sample may adhere to the wells of a microtiter plate. This limitation, however, can be overcome using a capture antibody unique to the specific test antigen to select it out of the serum, as illustrated in the sandwich technique below.

Sandwich ELISA

The sandwich technique is used to identify a specific sample antigen. The well surface is prepared with a known quantity of bound antibody to capture the desired antigen. After nonspecific binding sites are blocked using bovine serum albumin, the antigen-containing sample is applied to the plate. A specific primary antibody is then added that "sandwiches" the antigen. Enzyme-linked secondary antibodies are applied that bind to the primary antibody. Unbound antibody–enzyme conjugates are washed off. Substrate is added and is enzymatically converted to a color that can be later quantified. Canady *et al.* (2013) analyzed patient sera using the sandwich method to detect enhanced keratinocyte growth factor (KGF) levels in the sera of keloid and scleroderma patients compared to healthy controls to quantify human KGF (Figure 3).

One advantage of using a purified specific antibody to capture antigen is that it eliminates the need to purify the antigen from a mixture of other antigens, thus simplifying the assay and increasing its specificity and sensitivity.

Competitive ELISA

The key event of competitive ELISA is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody. First, the primary antibody is incubated with the sample antigen and the resulting antibody–antigen complexes are added to wells that have been coated with the same antigen. After an incubation period, any unbound antibody is washed off. The more antigen in the sample, the more primary antibody will be bound to the sample antigen. Therefore, there will be a smaller amount of primary antibody available to bind to the antigen coated on the well. Secondary antibody conjugated to an enzyme is added, followed by a substrate to elicit a chromogenic or fluorescent signal. Absence of color indicates the presence of antigen in the sample.

The main advantage of competition ELISA is its high sensitivity to compositional differences in complex antigen mixtures, even when the specific detecting antibody is present in relatively small amounts (Dobrovolskaia *et al.*, 2006). This method can be used to determine the potency of U.S. standardized allergen extracts (Dobrovolskaia *et al.*, 2006) and to measure the total antibodies to the capsular polysaccharide of *Haemophilus influenzae* type

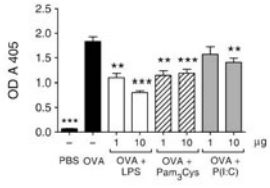

Figure 2. Indirect enzyme-linked immunosorbent assay (ELISA). Dermal exposure to Toll-like receptor ligands (lipopolysaccharide, Pam_3Cys, P(I:C)) was demonstrated to downregulate ovalbumin-specific IgE antibodies in serum, as measured by the indirect ELISA technique. Reprinted from Haapakoski *et al.* (2013).

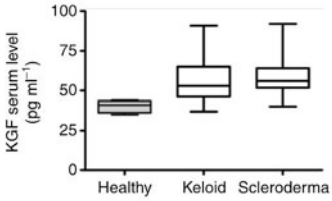

Figure 3. Sandwich enzyme-linked immunosorbent assay (ELISA). The "sandwich" method was used to detect enhanced keratinocyte growth factor (KGF) levels in the sera of keloid and scleroderma patients compared to healthy controls to quantify human KGF. Reprinted from Canady *et al.* (2013).

b in human sera from vaccinated subjects (Mariani *et al.*, 1998). Competitive ELISA is often used to detect HIV antibodies in the sera of patients. The HIV antigen is coated on the surface of the microtiter plate wells, and two specific antibodies are applied: one conjugated with enzyme and the other of the sera of the patient. Cumulative competition occurs between the two antibodies for the same antigen. If antibodies are present in the sera, then the antigen–antibody reaction occurs, leaving behind very low amounts of antigen available for binding with the enzyme-labeled antibody. Most of the unbound enzyme-labeled antibodies are washed off, producing minimal to no color change. Absence of color is indicative of an HIV-positive sample.

Multiple and portable ELISA

Multiple and portable ELISA is a new technique that uses a multicatcher device with 8 or 12 immunosorbent protruding pins on a central stick that can be immersed in a collected sample. The washings and incubation with enzyme-conjugated antigens and chromogens are performed by dipping the pins in prefilled microwells with reagents. The main advantage of these ready-to-use lab kits is that they are relatively inexpensive, can be used for large population screening, and do not require skilled personnel or laboratory equipment, making them an ideal tool for low-resource settings (Balsam *et al.*, 2013). Clinical applications include point-of-care detection of infectious diseases, bacterial toxins, oncologic markers, and drug screening.

SUMMARY

EIA/ELISA is a powerful method not only for general biomedical research but also as a diagnostic tool. It allows detection of all types of biological molecules at very low concentrations and quantities. Although it has its limitations, EIA/ELISA remains an important tool in both clinical and basic research, as well as in clinical diagnostics.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for journal club or other teaching exercises are available at http://dx.doi.org/10.1038/jid.2013.287.

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QUESTIONS

This article has been approved for 1 hour of Category 1 CME Credit. To take the quiz, with or without CME credit, follow the link under the "CME CREDIT" header.

1. Which of the following molecule(s) can be detected by ELISA?

A. Proteins.
B. Hormones.
C. Antibodies.
D. All of the above.

2. What does a weak color signal in competitive ELISA represent?

A. More antigen in the sample.
B. Less antigen in the sample.
C. Less antigen retained on the well.
D. Both a and c.

3. Which of the following is immobilized on the microtiter well in sandwich ELISA?

A. Detection antibody.
B. Sample.
C. Capture antibody.
D. Secondary antibody conjugated to an enzyme.

4. What is a major advantage of ELISA in comparison to other biological quantification techniques?

A. Detection of a molecule at a low concentration.
B. Inexpensive.
C. Low specificity.
D. Easily available.