ELISA

(Enzyme-Linked Immunosorbent Assay)

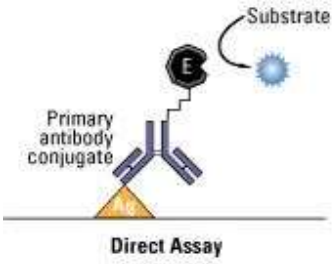
ELISA assays are similar in principle to RIAs but, instead of using antibodies or antigens conjugated to radioisotopes, they use antibodies or antigens covalently bound to enzymes. The conjugated enzymes are selected on the basis of their ability to catalyze the conversion of a substrate into a colored, fluorescent, or chemiluminescent product. These assays match the sensitivity of RIAs and have the advantage of being safer and, often, less costly.

The enzyme linked immunosorbent assay (ELISA) is a powerful method for detecting and quantifying a specific protein in a complex mixture. Originally described by Engvall and Perlmann (1971), the method enables analysis of protein samples immobilized in microplate wells using specific antibodies.

A number of variations of the basic ELISA assay have been developed (Figure 20-7). Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen.

Direct ELISA:

The direct detection method uses a primary antibody labeled with a reporter enzyme or a tag that reacts directly with the antigen. Direct detection can be performed with an antigen that is directly immobilized on the assay plate or with the capture assay format. Direct detection, while not widely used in ELISA, is quite common for immunohistochemical staining of tissues and cells.



Indirect ELISA:

Antibody can be detected, or its concentration determined with an *indirect ELISA* assay. Serum or some other sample containing primary antibody (Ab1) is added to an antigen coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the antibody bound to the antigen is detected by adding an enzyme-conjugated secondary antibody (Ab2) that binds to Ab1. Any free Ab2 is again washed away, and a substrate for the enzyme is added. The amount of colored, fluorescent, or luminescent reaction product that forms is measured using a specialized plate reader and compared with the amount of product generated when the same set of reactions is performed using a standard curve of known Ab1 concentrations. (A *direct* ELISA assay would detect the amount of antigen on the plate using enzyme coupled antibodies, and is rarely used.)

This version of ELISA is the method of choice to detect the presence of serum antibodies against *h*uman *i*mmunodeficiency *v*irus (HIV), the causative agent of AIDS.

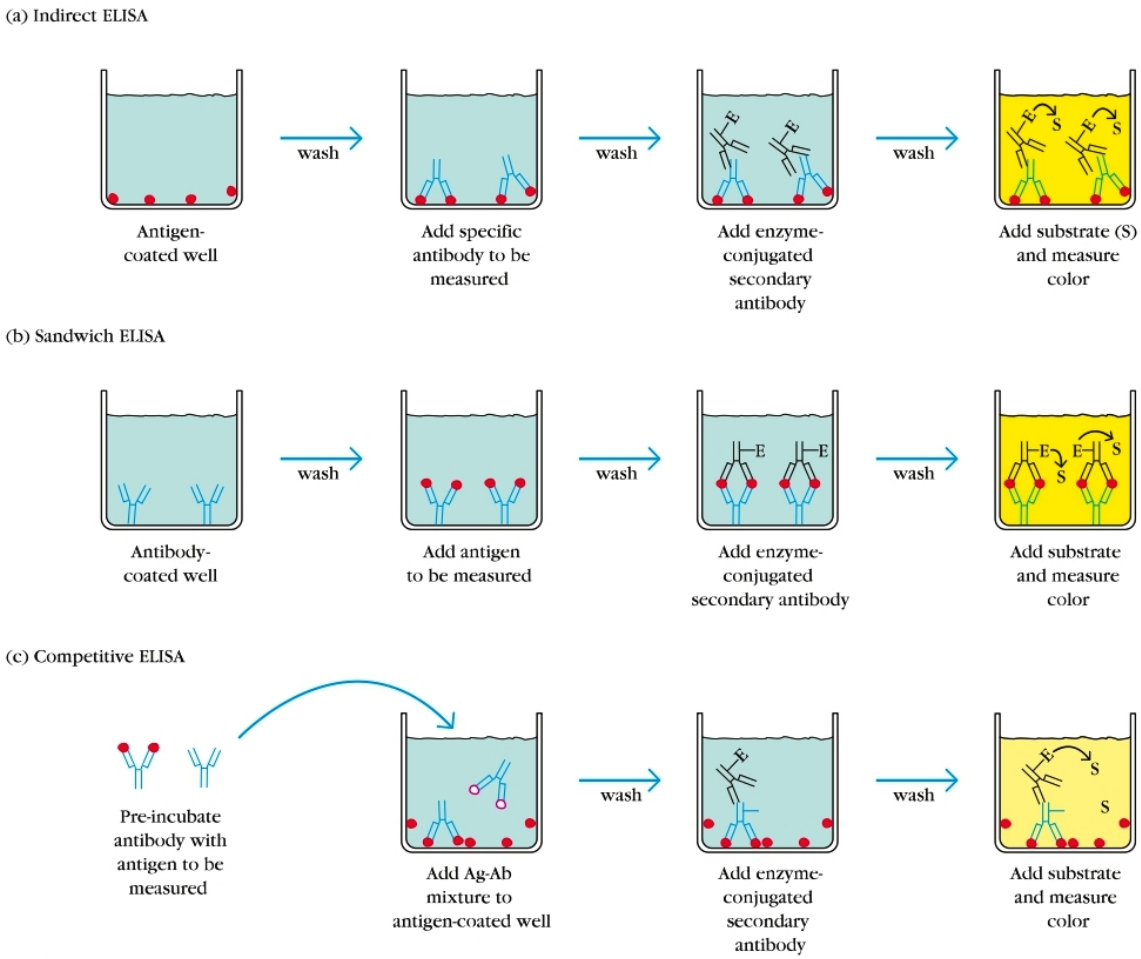


FIGURE 20-7 Variations in enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay that is identical in principle to the competition RIA described above, the concentration of antigen is inversely proportional to the color produced.

Sandwich ELISA:

Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing unknown amounts of antigen is allowed to react with the immobilized antibody. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

Sandwich ELISAs have proven particularly useful for the measurement of soluble cytokine concentrations in tissue culture supernatants, as well as in serum and body fluids.

Competitive ELISA:

The competitive ELISA provides another extremely sensitive variation for measuring amounts of antigen. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen-coated microtiter well. The more antigen present in the initial solution-phase sample, the less free antibody will be available to bind to the antigen-coated well. After washing off the unbound antibody, an enzyme-conjugated Ab2 specific for the isotype of the Ab1 can be added to determine the amount of Ab1 bound to the well. In the competitive assay, the higher the concentration of antigen in the original sample, the lower the final signal.

Applications of ELISA:

- Serum Antibody Concentrations
- Detecting potential food allergens (milk, peanuts, walnuts, almonds and eggs)
- Disease outbreaks – tracking the spread of disease.
    - E.g. HIV, bird flu, common colds, cholera, STD etc
- Detections of antigens
    - E.g. pregnancy hormones, drug allergen, GMO, man cow disease
- Detection of antibodies in blood sample for past exposure to disease
    - E.g. Lyme disease, trichinosis, HIV, bird flu.