Introduction to Immunoassays

ELISA Technique

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Introduction to Immunoassays
ELISA Technique
After completion of this lecture, you will be able to:

• define immunoassay
• describe the structure and preparation of antibodies
• define four categories of immunoassay methodology
• *(competitive and noncompetitive, and homogeneous and heterogeneous)*

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Introduction

• An immunoassay is a test that uses antibody and antigen complexes as a means of generating a measurable result.

• An antibody:antigen complex is also known as an immuno-complex.
“Immuno” & “assay”

- “Immuno” refers to an immune response that causes the body to generate antibodies,

  and

- “assay” refers to a test. Thus, an immunoassay is a test that utilizes immunocomplexing when antibodies and antigens are brought together.

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• **Immunoassays** are different from other types of laboratory tests, such as **colorimetric tests**, because they use **antibody:antigen** complexes to generate a signal that can be measured.

• **In contrast**, most routine clinical chemistry tests utilize chemical reactions between the reagent (a solution of chemicals or other agents) and patient sample to generate a test result.
• **An antibody** is a protein that is produced by the body in response to an “invading” (foreign) substance.

• **Antibodies** are produced as part of the body’s immune response to protect itself. For instance, some immunoassays test for the presence of antibodies to cancer molecules. Thus, if the antibodies are present, it means invading cancer cells are, too.

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An antigen

- An antigen is the substance that the body is trying to “fight off” (eliminate or reduce) by mounting an immune response. Some immunoassays test for antigens directly, rather than looking for the antibodies. In a test to measure the concentration of a therapeutic drug, for example, the drug is the antigen that binds to the antibody.
An analyte

- An analyte is anything measured by a laboratory test. In immunoassay testing, the analyte may be either an antibody, or an antigen.
**Immunoassays**

- Immunoassays utilize one or more select antibodies to detect analytes of interest. The analytes being measured may be those that are naturally present in the body (such as a thyroid hormone), those that the body produces but are not typically present (such as a cancer antigen), or those that do not naturally occur in the body (such as an abused drug).

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Antibodies

- Antibodies possess high
  a) specificity
  and
  b) affinity

  for a specific antigen. It is the specific binding of an antibody to an antigen that allows the detection of analytes by a variety of immunoassay methods.
Structure of Antibodies

• Antibodies (Ab) are a type of protein called immunoglobulins.
• The most common one is immunoglobulin G (IgG).
• IgG is a protein composed of two main structural and functional regions.
**Fab region**: Contains the antigen (Ag) binding site that varies between different antibodies.

**Fc region**: Region of constant structure within an antibody class.
Preparation of Polyclonal and Monoclonal Antibodies

- Antibody reagents are developed from either polyclonal or monoclonal antibodies.

- Polyclonal antiserum (serum from blood containing the desired antibodies) is generated in animals, most commonly sheep, rabbits, or goats.
Cont’ed

• **The animals** produce the antiserum - just as a human would - as a defense mechanism when exposed to an antigen.

• **Antiserum** contains a mixture of *antibodies*, each of which may bind to different antigen binding sites, or epitopes.
Producing antiserum

• The process of making an antiserum begins by injecting a solution that contains the antigen of interest into an animal. This antigen of interest is sometimes called an immunogen, because it can stimulate an immune response.

Over time, and in some cases with multiple injections, the immune system of the animal produces antibodies to the antigen that was injected.

Blood is collected from the animal, and serum is isolated from the blood. This serum is usually rich in antibodies that recognize the antigen, and is called the antiserum.

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Figure 1-2  Multiple antigen specificities of polyclonal antibodies
polyclonal antibodies

• Antiserum usually contains a mixture of antibodies that recognize and bind to the same antigen, but they may attach to different epitopes (see Figure 1-2). An antigen that has multiple sites for antibodies to bind is called a multivalent antigen. These types of antibodies, present as a diverse mixture, are called polyclonal antibodies.
Monoclonal antibodies

- Monoclonal antibodies differ from polyclonal antibodies in that they are highly specific for a single epitope on a multivalent antigen (see Figure 1-3). They are produced from a single cell line using hybridoma technology and mouse myeloma cell lines.

- Hybridomas are antibody-producing tumor cells that produce many copies of the same antibody and grow easily in laboratory cell culture.

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An advantage

• An advantage of monoclonal antibodies is that the hybridoma cell line that produces them is potentially “immortal” and can produce the same antibodies consistently and indefinitely.

• A polyclonal antisera produced by immunization of animals can vary from animal to animal, and a useful antiserum may no longer be available if the single animal that produces it dies.
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Figure 1-3 Uniform specificities of monoclonal antibodies
Hybridomas are produced in a multi-step procedure (see Figure 1-4):

1. Injecting a specific antigen into a host animal (typically a mouse);
2. Isolating antibody-producing cells (B cells) from the spleen of the mouse;
3. Fusing these B cells with a specific type of tumor cell that grows easily in culture and produces antibodies;
4. Isolating successful hybridomas (fused cells) that produce antibodies specific for the antigen of interest.
Figure 1-4  Procedure for generating monoclonal antibodies
In immunoassays

- In immunoassays, both monoclonal and polyclonal antibodies are used for detecting antigens, each with specific strengths for particular applications.
- Immunoassays that detect antibodies in patient sera are likely to involve detection of polyclonal antibodies generated by the patient’s immune system.
Categories of Immunoassay Methodologies

- The immunoassay methodologies are:
- noncompetitive and competitive immunoassays,
  and
- homogeneous and heterogeneous immunoassays
labeled material

- All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present.
- A label is a molecule that will react as part of the assay, so a change in signal can be measured in the blood:reagent solution.
Examples of a label

- Examples of a label include a radioactive compound, an enzyme that causes a change of color in a solution, or a substance that produces light.

- The label can be applied during the manufacture of the reagent to either the antibody (Ab*, see Figure 1-5) or antigen (Ag*, see Figure 1-6).

- Immunoassay technologies utilize different formats to distinguish the bound antigen-antibody complex from the free unbound label.
**Figure 1-5** Labeled antibodies allow detection of antigen/antibody complexes in immunoassays

**Figure 1-6** Labeled antigen also allows detection of antigen/antibody complexes in immunoassays
The measurement of analyte in an immunoassay is achieved by using either a competitive or a noncompetitive format.
Competitive Format

• In competitive formats,
• unlabelled analyte (usually antigen) in the test sample is measured by its ability to compete with labeled antigen in the immunoassay.
• The unlabeled antigen blocks the ability of the labeled antigen to bind because that binding site on the antibody is already occupied.
Con’ted

• Thus, in a **competitive immunoassay**, less label measured in the assay means more of the **unlabeled (test sample) antigen** is present. The amount of antigen in the test sample is inversely related to the amount of label measured in the

• competitive format (Figure 1-7).
One step competitive format

- In the one step competitive format (see Figure 1-8), both the labeled antigen reagent \((\text{Ag}^*)\) and the unlabeled specimen (or test sample analyte) compete for a limited amount of antibody.
Two step competitive format

• In the two step competitive format, the antibody concentration of the reaction solution is present in excess in comparison to the concentration of antigen. Antibody reagent is first incubated with specimen containing antigens of interest; then in the second step, labeled antigen is added. Remember that in the competitive format, less bound labeled antigen indicates more antigen present in the test sample. Two step competitive assay formats provide several fold improved assay sensitivity compared to one step assay formats.
Figure 1-9  Two step competitive immunoassay
• **Noncompetitive assay** formats generally provide the highest level of assay **sensitivity** and **specificity** and are applied to the measurement of critical analytes such as cardiac and hepatitis markers. This format is referred to as a “**sandwich**” assay because analyte is bound (**sandwiched**) between two highly specific antibody reagents (Figure 1-10).  

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Sandwich Assays: Antibodies bind to two sites on analyte

Solid support:
- microparticles
- beads
- microtiter plates

Figure 1-10 Noncompetitive sandwich method of immunoassay
**Noncompetitive assay**

- Noncompetitive assay formats can also utilize either one **step or two step methods**, as with the competitive assay.
- The two step assay format employs wash steps in which the sandwich binding complex is isolated and washed to remove excess unbound labeled reagent and any other interfering substances.
- The two step noncompetitive format usually offers the highest specificity and sensitivity of all the assay formats discussed here.

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In noncompetitive assays, the measurement of labeled analyte, usually antibody, is directly proportional to the amount of antigen present in the sample. This can be represented by a dose response curve (Figure 1-11). The X-axis plots concentration of an antigen. The Y-axis plots response, which in this case is signal. Thus, the more antigen that is present, the more labeled antibody that will bind. This direct proportionality is in contrast with the indirect proportionality of competitive immunoassays discussed earlier.
Homogeneous and Heterogeneous Immunoassay Methods

• Immunoassay methods that require separation of bound Ab-Ag* complex are referred to as heterogeneous immunoassays. Those that do not require separation are referred to as homogeneous immunoassays.

• Homogeneous methods have been generally applied to the measurement of small analytes such as abused and therapeutic drugs. Since homogeneous methods do not require the separation of the bound Ab-Ag* from the free Ag*, they are generally much easier and faster to perform.
**Figure 1-12** Homogeneous and heterogeneous immunoassays
Summary

• **Immunoassays** are tests that use *antibody* and *antigen* complexes (also called *immunocomplexes*) to measure the presence of a specific analyte in a sample.

• **Antibodies** are proteins that are normally produced by the immune system in response to a foreign substance.

• **Antigens** are the molecules that antibodies bind to, which in the body could be an invading pathogen, or the foreign molecules injected into an animal to trigger the immune response.

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Summary

- Antibodies are comprised of two major regions, the **Fab** region (antigen specific) and the **Fc** region.
- Antibody preparations are either **polyclonal antisera**, which recognize multiple sites on antigens, or **monoclonal antibodies**, which recognize single sites on antigens.
Summary

• In immunoassays, the antibody or antigen is labeled in order to have a measurable signal that corresponds to the concentration of the analyte.

• Immunoassays can be either competitive or noncompetitive. In competitive immunoassays, the amount of antigen is indirectly proportional to the amount of signal. In noncompetitive immunoassays, the amount of antigen is directly proportional to the amount of signal.
Summary

- Homogeneous immunoassays do not require separation of unbound complexes from the bound complexes, and thus are faster and easier to perform than heterogeneous immunoassays.

- Heterogeneous immunoassays require the separation of unbound complexes, often utilizing a solid phase reagent such as a magnetic particle or plastic bead.
Questions?

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