Microtitter ELISA Basics

SCST Superworkshop - Immunoassay Workshop
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Iowa State Seed Science Center
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ELISA History

- 1798 - First demonstration of vaccination smallpox vaccination (Edward Jenner)
- 1900 - Antibody formation theory (Paul Ehrlich)
- 1938 - Antigen-Antibody binding hypothesis (John Marrack)
- 1948 - Antibody production in plasma B cells
- 1959-1962 - Discovery of antibody structure
- 1960 - Radioimmunoassay was first described in a scientific paper by Rosalyn Sussman Yalow and Solomon Berson published in 1960
- 1966 - A technique to prepare something like immunosorbent to fix antibody or antigen to the surface of a container was published by Wide and Jerker Porath in 1966
- 1971 - Peter Perlmann and Eva Engvall at Stockholm University invented ELISA, **Enzyme-Linked Immunosorbent Assay**
- 1975 - Generation of the first monoclonal antibodies (George Kohler and Cesar Milstein)

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Agenda

- Definitions
- ELISA Benefits
- Basic ELISA Principle
- Product Development Process
- Antibodies
- Enzyme Labels
- Substrates
- Plate Washing
- Different Types of ELISA
- Q & A

Definitions

Enzyme Linked Immuno-Sorbent Assay

- ELISA – an immunological test, using enzyme as a label to determine presence of target protein
- The enzyme linkage or labeling allows you to follow your target protein and if present (qualitative) and at what amounts (quantitative)
- An enzyme conjugate is an enzyme bound or joined with an antibody which binds with your target protein. This enzyme labeling is a safe and effective way to track your antibody

Definitions

Antigen

- Any substance that stimulates an immune response
- The antigen is your target protein which comes from your sample extract
  - Bt protein in corn samples, the antigen binds to the antibody

Epitope

- The region on an antigen to which an antibody binds

ELISA Benefits

- Sensitivity
- Reliability
- Affordability
- High Throughput
- Quick turnaround
- Relative ease of use
- Qualitative and quantitative results

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Basic ELISA Principle

- Detection of an "analyte"
- Liquid sample is added to a solid phase
- Multiple liquid reagents are sequentially added
- Incubated and washed
- Color development
- Read the absorbance value

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Product Development Process

- Clear definition
- Qualitative or quantitative results
- Required sensitivity
- Limit of detection
- Cross reactivity with other antigens
- Sample matrix
- Time to completion

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Antibodies

- **Polyclonal** - Represents a collection of antibodies from different B cells that recognize multiple epitopes on the same antigen. Each of these individual antibodies recognizes a unique epitope that is located on that antigen
- **Monoclonal** - A Monoclonal antibody, by contrast, represents antibody from a single antibody producing B cell and therefore only binds with one unique epitope.
- Purified
  - Protein A or G affinity chromatography
- Purity of immunogen is key
- Screening clones for specificity and sensitivity

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Comparison of Polyclonal and Monoclonal antibodies

- **Polyclonal antibodies**
  - Inexpensive to produce
  - Time scale is short
  - Produces large amounts of non-specific antibodies
  - Recognizes multiple epitopes on any one antigen
  - Can have batch-to-batch variability
  - Highly stable and tolerant of pH or buffer changes
- **Monoclonal antibodies**
  - Expensive to produce
  - Time scale is long for hybridomas
  - Can produce large amounts of specific antibodies
  - Recognizes only one epitope on an antigen
  - Once a hybridoma is made, it is a constant and renewable source
  - No or low batch-to-batch variability
  - More sensitive to pH and buffer conditions

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Enzyme Labels

- horseradish peroxidase (HRP)
- alkaline phosphatase (AP)
- enzymes allow for detection because they produce an observable color change in the presence of certain substrates

Substrates

- **3,3’,5,5’-Tetramethylbenzidine** or **TMB**
  - TMB is degraded by sunlight and by fluorescent lights
- *p*-nitrophenylphosphate (pNPP)

Plate Washing

Poor plate washing technique is a major cause for low precision ELISA

- **Qualitative** - less precision needed. However, some higher sensitivity or semi- quantitative ELISA can require a high precision level
- **Quantitative** – maximum precision possible

Wash Buffer

- Detergents – removes non target proteins
- Blocking – blocks desorption of target proteins
- Higher concentration of wash buffer (detergents) – poorly mixed buffers could lead to higher removal of target protein

Plate Washing Tips

- Follow vendor guidelines
- Not all test are the same
- Completely fill wells
- Rotate plate at midway point of wash cycle
- Tap plates on paper towel but do not allow wells to dry completely
- Keep plates upside down on moist towel until ready for substrate

Plate Reading

- When TMB reacts with HRP, a blue color appears and the color change can be read on a spectrophotometer at a wavelength of 650 nm
- When pNPP reacts with AP, a yellow color appears and the color change can be read at 405 nm on a spectrophotometer

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Types of ELISA

- **Direct ELISA**
- (1) Direct ELISAs involve attachment of the antigen to the solid phase, followed by an enzyme-labeled antibody. This type of assay generally makes measurement of crude samples difficult, since contaminating proteins compete for plastic binding sites.

Types of ELISA

- **Indirect ELISA**
- (2) Indirect ELISAs also involve attachment of the antigen to a solid phase, but in this case, the primary antibody is not labeled. An enzyme-conjugated secondary antibody, directed at the first antibody, is then added

Types of ELISA

- **Competitive ELISA**
- (3) The third type of ELISA is the Competition Assay, which involves the simultaneous addition of 'competing' antibodies or proteins. The decrease in signal of samples where the second antibody or protein is added gives a highly specific result.

Types of ELISA

- **Sandwich ELISA**
- (4) The last type of assay is the sandwich ELISA. Sandwich ELISAs involve attachment of a capture antibody to a solid phase support. Samples containing known or unknown antigen are then added in a matrix or buffer that will minimize attachment to the solid phase. An enzyme-labeled antibody is then added for detection.
- There are indeed many variations to this method. ELISAs are adaptable to high-throughput screening because results are rapid, consistent and relatively easy to analyze. The best results have been obtained with the sandwich format, utilizing highly purified, prematched capture and detector antibodies. The resulting signal provides data which is very sensitive and highly specific.

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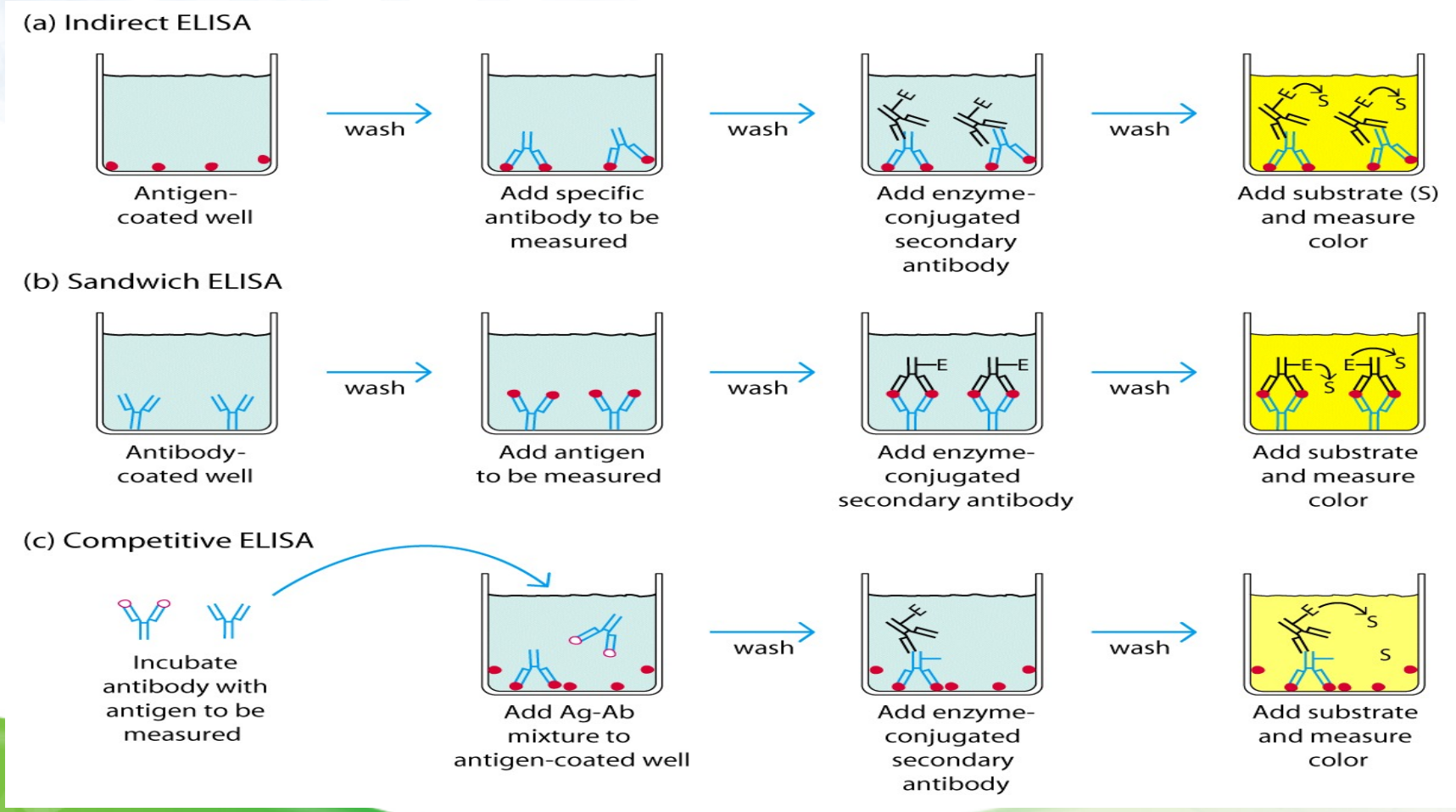
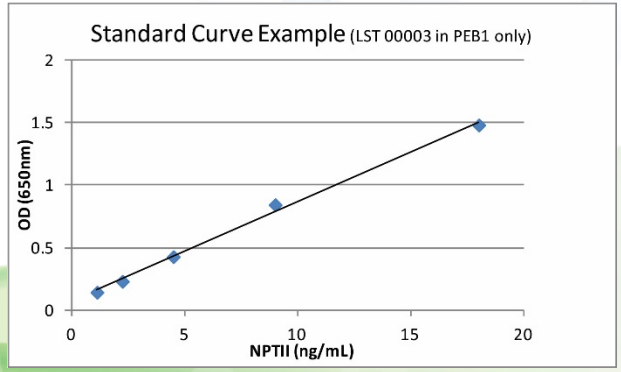
Types of ELISA



- https://www.youtube.com/watch?v=nNjlBCnpGZ4

Types of ELISA

- **Qualitative** results provide a simple positive or negative result for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation is often used to distinguish positive and negative samples
- **Quantitative**
  - Known concentrations of antigen are used to produce a standard curve and then this data is used to measure the concentration of unknown samples by comparison to the linear portion of the standard curve
  - This can be done directly on the graph or with curve fitting software which is typically found on ELISA plate readers



ELISA Interference

- **Hook Effect -** The **hook effect** or the **prozone effect** is a false negative result with certain immunoassays due to very high concentrations of a particular analyte