Enzyme-linked immunosorbent assay (ELISA)

Sarah Cheah
March 6th, 2020
Outline

- Introduction
- History of ELISA
- General Principles of ELISA
- Application of ELISA
- Current development on ELISA
What is ELISA

- A type of immunoassay used to detect and measure certain target molecule in a mixture solution
- Qualitative (detect presence) or/and Quantitative (what is the level of molecule present)
- Based on antibody-antigen reaction, they bind together to form complex
- The complex is then labelled with enzyme-linked antibody which produce colored product upon addition of substrate


Some history of ELISA ....RIA

- Radioimmunoassay (RIA) was developed in 1960 by Berson and Yalow.
- Radio-labelled insulin is used to measure the concentration of insulin in human plasma.
- Based on competition between labelled insulin and unlabeled (natural) insulin.
- The more natural insulin, the less radiolabeled insulin bind to the antibody.
- The radiation level is inversely proportional to the level of natural insulin in the human plasma.

# Advantages and Drawbacks of RIA

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precise</td>
<td>Short-life reagents</td>
</tr>
<tr>
<td>Very sensitive</td>
<td>Expensive equipment</td>
</tr>
<tr>
<td></td>
<td>Health concerns</td>
</tr>
</tbody>
</table>

General principle of ELISA

- Solid phase is needed => 96 well plate
- Antigen or Primary Antibody (liquid phase) is immobilized on the solid phase
- Add sample containing analyte of interest
- Enzyme-linked secondary antibody is added (depends on type of ELISA)
- Types of enzymes used: 1. Alkaline Phosphates (substrate: p-nitrophenyl phosphate)
  2. Horseradish Peroxidase (substrate: chromogenic substrate/chemiluminescent substrates)
- The reaction usually takes 30-60min
- Add HCl/NaOH (stop solution) to stop the enzyme activity and stop the reaction
- The products are detected using a microtiter plate reader

Direct ELISA

- Target antigen is immobilized on solid phase
- Primary antibody/antigen is enzyme-linked
- Secondary antibody is not involved
- For qualitative analysis of macromolecules
- Analyze the immune response to antigen

- **Pros**: less prone to errors (less reagent/steps)
- **Cons**: - no signal amplification (less sensitive)
  - antigen/antibody immobilization not specific (higher background noise)

Indirect ELISA

- Target antigen is immobilized on solid phase
- Sample containing primary antibody against target antigen added
- Secondary enzyme-linked antibody against primary antibody added
- To find presence of antibody in antisera following exposure to disease (vaccination)

**Pros:**
- Highly sensitive
- More flexibility

**Cons:**
- Longer procedure
- Secondary antibody may cross-react

Competitive ELISA

- Competitive interaction between target molecule in sample and enzyme-linked molecule
- Target antibody, antigen that is immobilized on the solid phase
- Add sample mixture to the wells
- Add enzyme-linked antibody to the wells
- With increasing amount of natural antibody, the signal decrease
- Used to measure the concentration of target molecule

- **Pros:**
  - easy, no secondary antibody required
  - no sample preparation required
- **Cons:**
  - labelling the antibodies may render its inactivation

Sandwich ELISA

- Immobilization of capture antibody on the solid phase
- Antigen of interest in sample mixture will bind to capture antibody
- Detection antibodies is added and will bind to antigen at a different epitope
- Direct or indirect Sandwich ELISA

- Pros: - highly sensitive
  - highly specific
- Cons: - expensive
  - labor-intensive

Application of ELISA

- Disease diagnostic (HIV, cancer, viral infection, food allergy)
- Detection and quantitation of antibodies in response to infection (vaccine design)
- Detection and quantitation of antigens of interest (Pregnancy kit)
- Detect allergen in commercial products
- Research laboratories
- Test for crop allergens


https://en.wikibooks.org/wiki/Methods_and_Concepts_in_the_Life_Sciences/Immunoassays
Influenza A & B => annual epidemics in human race due to antigenic change at hemagglutinin (HA) and neuraminidase (NA) antibody binding site (antigen drift)

Problems:
- Composition of vaccines needs to be updated every year (takes 6-8 months)
- Current available influenza vaccine not able to protect against emerging influenza virus

Universal Vaccine: vaccine against stalk domain (HA2) of HA

Pros: - avoid the need of annual re-formulation and re-administration of vaccine
- allow time for more effective vaccine to be made in event of pandemic

https://www.vanderbilt.edu/vicb/DiscoveriesArchives/targeting_flu_through_host_protein.html


Influenza Anti-Stalk Antibodies: Development of a New Method for the Evaluation of the Immune Responses to Universal Vaccine. Vaccines 2020, 8, 43.
Results

- Competitive ELISA => able to detect stalk specific antibodies
- Standard serological assay => insufficient to detect stalk-specific antibodies accurately (stalk-induced antibodies have various functions)
- These methods could only detect HA1 domain
- Limitation: not able to tell if the antibodies bound are functional (Need to couple with other assay, not able to tell whether antibodies detected are alive or dead)

## Advantages and Disadvantages of ELISA

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple procedure</td>
<td>Labor-intensive</td>
</tr>
<tr>
<td>High specificity and sensitivity</td>
<td>Antibody is expensive</td>
</tr>
<tr>
<td>High efficiency</td>
<td>Possibility of false positive/negative</td>
</tr>
<tr>
<td>Simultaneous analysis can be performed</td>
<td>Antibody instability</td>
</tr>
<tr>
<td>Safe and eco-friendly</td>
<td>Antibody needs to be refrigerated</td>
</tr>
<tr>
<td>Cost-effective</td>
<td></td>
</tr>
</tbody>
</table>

Current development of ELISA

- Automation of ELISA assays (Agilent Bravo Platform)
  - coating/delivery of reagent/plate washing
  - to reduce time and steps
- Instant ELISA
  - add sample to pre-coated ELISA
  - one hour, one wash
- New technique? CyVek Analyzer
  - just add sample into cartridge & go!

https://www.ddw-online.com/screening/p191009-enzyme-linked-immunosorbent-assays-(elisa)-recent-innovations-take-analyte-detection-to-new-levels.html