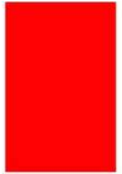
ELISA

CONTENTS

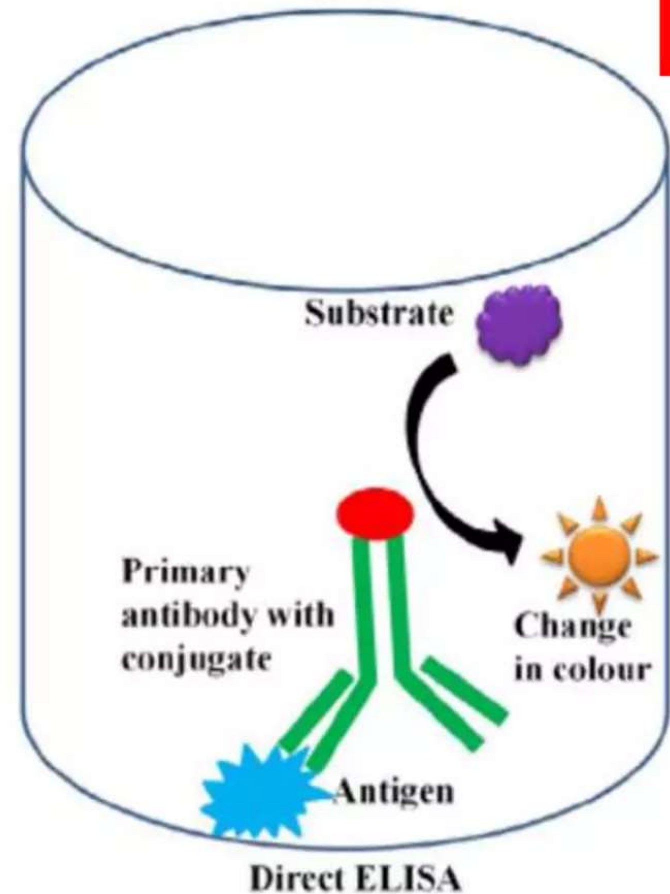
- Introduction
- Principle of ELISA
- General procedure
- Requirements
- Types of ELISA
- ELISA data interpretation
- Applications
- Reference



INTRODUCTION

- Enzyme-linked immunosorbent assay (ELISA) is a labeled immunoassay that is considered the gold standard of immunoassays.
- This immunological test is very sensitive and is used to detect and quantify substances, including antibodies, antigens, proteins, glycoproteins, and hormones.
- The detection of these products is accomplished by complexing antibodies and antigens to produce a measurable result.
- Two different research teams invented the direct ELISA simultaneously by scientists Eva Engvall and Peter Perlman and by Van Weemen and Schuurs in 1971.

- The ELISA was developed by the modification of the radioimmunoassay (RIA).
- Ag- Ab interaction is utilized in ELISA testing and allows for identifying specific protein antibodies and antigens, with only small amounts of a test sample.
- ELISA testing is used to diagnose HIV infection, pregnancy tests, and blood typing, among others.



Direct ELISA

Principle of ELISA

- ELISA is a plate-based assay technique. Along with the enzyme-labelling of antigens or antibodies, the technique involves following three principles in combination which make it one of the most specific and sensitive than other immunoassays to detect the biological molecule:

1. An immune reaction i.e. antigen-antibody reaction.

2. Enzymatic chemical reaction i.e. enzyme catalyses the formation of colored (chromogenic) product from colorless substrate.

3. Signal detection and Quantification i.e. detection and measurement of color intensity of the colored products generated by the enzyme and added substrate.

General procedure

There are four main general steps to completing an ELISA immunoassay. These steps are:

- ➢Coating (with either antigen or antibody)
- ➢Blocking (typically with the addition of bovine serum albumin [BSA])
- ➢Detection
- ➢Final read

COATING

Polystyrene plate is treated with a solution of either antigen or antibody.

remove liquid and wash plate

BLOCKING

An unrelated protein-based solution is used to cover all unbound sites on the plates

remove liquid and wash plate

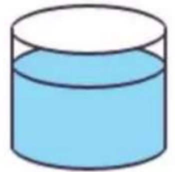
DETECTION

Enzyme-conjugated antibody or antigen binds specifically to the target antigen or antibody

remove liquid and wash plate

READ RESULTS

Substrate is added and the signal produced by the enzyme-substrate reaction is measured



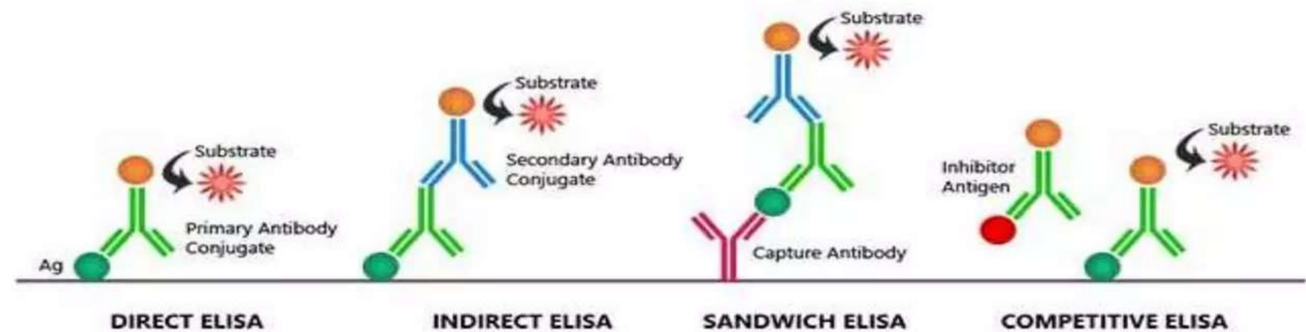
Requirements

1. Testing sample (serum , saliva, urine, CSF)
2. Blocking agents(addition of bovine serum albumin [BSA])
3. Antibodies (1°,2°)
4. Micro titre plate (polystyrene plates, typically in 96-well)
5. Washing buffers(phosphate-buffered saline (PBS) and a non-ionic detergent, to remove unbound material)
6. Enzymes (horseradish peroxidase , alkaline phosphatase)
7. Substrates/ chromogens (for HRP is hydrogen peroxide and results in a blue color change)

Types of ELISA

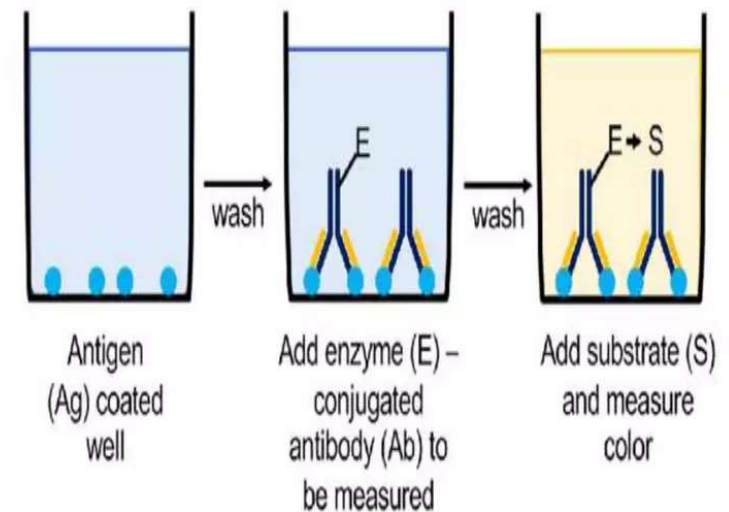
In general, ELISAs can be grouped into the four main categories:

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA



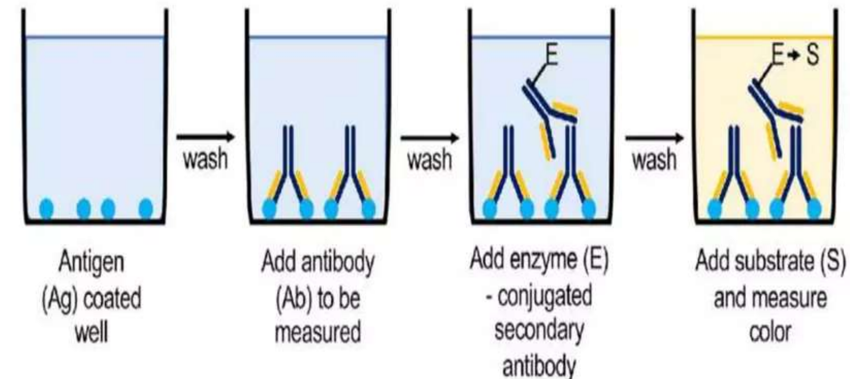
Direct ELISA

- Direct ELISA detection is much faster than other ELISA techniques as fewer steps are required. The assay is also less prone to error since fewer reagents and steps are needed, i.e. no potentially cross-reacting secondary antibody needed.
- The advantages of using direct ELISA include eliminating secondary antibody cross-reactivity, and due to fewer steps, it is rapid compared to indirect ELISA.
- Its disadvantages include its low sensitivity compared to the other types of ELISA and its high cost of reaction.



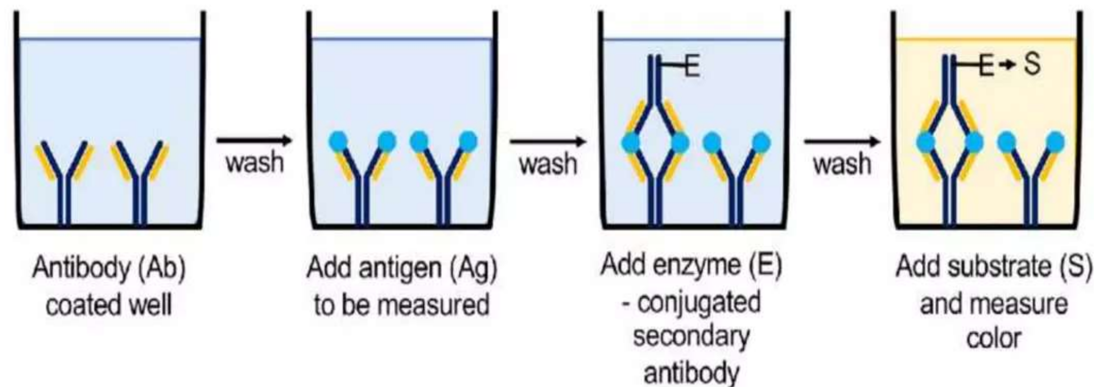
Indirect ELISA

- Detection is a two-step process. First, an unlabeled primary antibody binds to the specific antigen. Second, an enzyme conjugated secondary antibody that is directed against the host species of the primary antibody is applied.
- The indirect ELISA method has high sensitivity since more than one labeled secondary antibody can bind the primary antibody;
- Indirect ELISA assays take longer to run than direct ELISAs since an additional incubation step for the secondary antibody is required. The indirect ELISA is most suitable for determining total antibody concentration in samples.



Sandwich ELISA

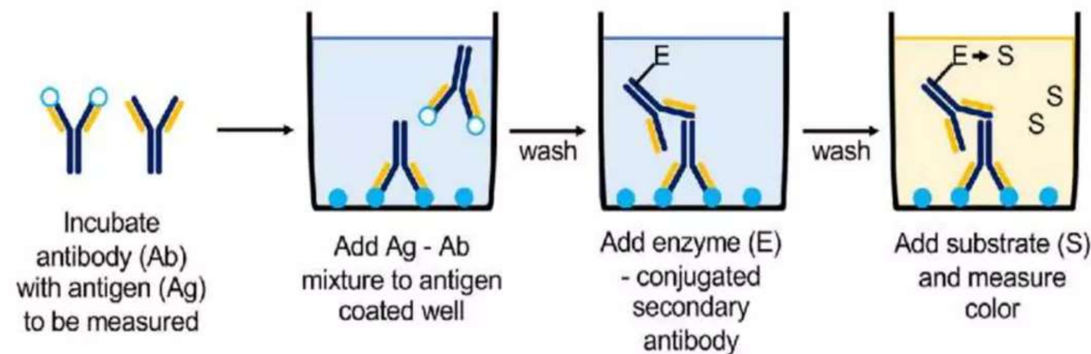
- Sandwich ELISA begins with a capture antibody coated onto the wells of the plate. It is termed a "sandwich" because the antigens are sandwiched between two layers of antibodies (capture and detection antibodies).
- Sandwich ELISAs require the use of matched antibody pairs (capture and detection antibodies)



- It is important that matched antibody pairs are tested specifically in sandwich ELISA to ensure that they detect different epitopes, to achieve accurate results. The capture antibody, as its name implies, binds the antigen that can then be detected in a direct ELISA or in an indirect ELISA configuration.
- High sensitivity - 2-5 times more sensitive than direct or indirect ELISA
- High specificity - two antibodies are involved in capture and detection
- Flexibility - both direct and indirect detection can be used

Competitive ELISA

- The competitive ELISA tests for the presence of an antibody specific for antigens in the test serum. This type of ELISA utilizes two specific antibodies, an enzyme-conjugated antibody and another antibody present in the test serum (if the serum is positive).
- This type of ELISA depends on the competitive reaction between the sample antigen and antigen bound to the wells of microtiter plate with the primary antibody.
  First, the primary antibody is incubated with the sample.



- This results in the formation of Ag-Ab complex which are then added to the wells that have been coated with the same antigens. After an incubation, unbound antibodies are washed off. The more antigen in the sample, more primary antibody will bind to the sample antigen.
- Therefore there will be smaller amount of primary antibody available to bind to the antigen coated on well. Secondary antibody conjugated to an enzyme is added, followed by a substrate to elicit a chromogenic signal. **Concentration of color is inversely proportional to the amount of antigen present in the sample.**

ELISA data interpretation

- In the ELISA protocol, usually, a serial dilution of concentrations is placed in the wells of the plate. After the results are measured, a standard curve from the serial dilutions data is plotted with a concentration on the x-axis using a log scale and absorbance on the y-axis using a linear scale.
- The ELISA assay yields two different types of data output:
- **Qualitative**
- **Quantitative**

Nature of results in ELISA

Qualitative

Determines the presence of Ag , Ab , proteins, peptides or hormones. ELISAs can also be used to achieve a yes or no answer indicating whether a particular antigen is present in a sample, as compared to a blank well containing no antigen or an unrelated control antigen

Quantitative

ELISA data can be interpreted in comparison to a standard curve (a serial dilution of a known, purified antigen) in order to precisely calculate the concentrations of antigen in various samples.

Applications

Enzyme-linked immunosorbent assays are applied in many diagnostic tests. Some of the uses of ELISA can include the following:

- **Detect and Measure the Presence of Antibodies in the Blood**
- Antibodies against infectious disease (antibacterial, antiviral, antifungal)
- Hepatitis A, B, C, HIV, etc.
- **Detect and Estimate the Levels of Tumor Markers**
- Prostate-specific antigen (PSA)
- Carcinoembryonic Antigen (CEA)

- **Detect and Estimate Hormone Levels**
  - Luteinizing hormone
- Follicular stimulating hormone
- Prolactin
- Testosterone
- Human chorionic gonadotropin (hCG)
- **Screening Donated Blood for Possible Viral Contaminants**
- anti-HIV-1/2
- anti-HCV
- HBsAg
- **Detecting Drug Abuse**
- Amphetamine,
- Methamphetamine
- 3,4-methylenedioxymethamphetamine
- Cocaine
- Benzoylecgonine

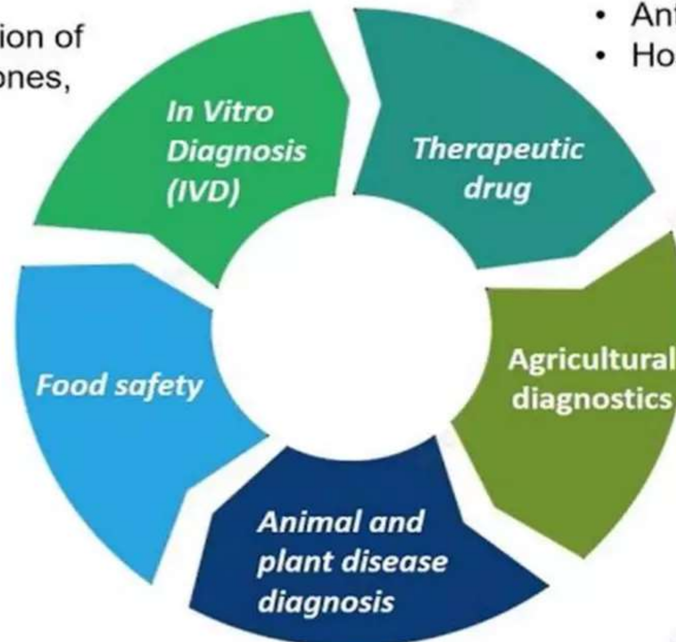
- Cholera
- HIV
- Influenza
- Covid 19
- **Detecting Past Exposures**
- HIV
- Lyme disease
- Hepatitis

Application of ELISA kit

GenScript
Make Research Easy

- Detection and quantification of antigens, antibodies, hormones, and other molecules.

- Pharmacokinetic (PK)
- Anti-Drug Antibody (ADA)
- Host cell proteins (HCP)



- Testing of soil, water, seeds, compost, manure, biosolids, and plant tissue samples
- Ensure the safety of the samples against hazardous contaminants such as pathogens, toxins, pesticides, and heavy metals
- Evaluation of quality, composition, and suitability as per the quality of output to be derived from fields.

- Food labeling
- Food hygiene
- Food additives
- Pesticide residues

- Diagnosis of animal/ plant diseases and research on biotic pathogens and host pathogen et.al