

Enzyme-linked immunosorbent assay (ELISA)

Microbiology V

Enzyme-linked immunosorbent assay (ELISA)

- Enzyme-linked immunosorbent assay (ELISA) utilizes an enzyme system to show specific combination of an antigen with its antibody.
- It is a method of quantifying an antigen immobilized on a solid surface.
- ELISA uses a specific antibody with a covalently coupled enzyme.
- The amount of antibody that binds the antigen is proportional to the amount of antigen present
- It is determined by **spectrophotometrically** measuring the conversion of a clear substance to a **colored product** by the coupled enzyme.

- The ELISA technique was first conceptualized and developed by **Peter Perlmann** and **Eva Engvall** at Stockholm University, Sweden.

Components of ELISA:

- The ELISA test involves an enzyme.
- It also involves an antibody or antigen.

- Enzyme system of ELISA consists of:
 - an **enzyme** labeled to a specific antibody or antigen and
 - a **chromogenic substrate** which is added after antigen-antibody reaction.
- The **substrate** is hydrolysed by the **enzyme** attached to antigen-antibody complexes.
- An ELISA test uses components of the immune system (IgG or IgM antibodies) and chemicals for the detection of immune responses in the body.

- The antigen or antibody is coated on solid surface such as in plastic tube or well of microtiter plate.
- After the antigen-antibody complex is formed they remain firmly attached to solid surface during subsequent washing stages.
- Following the antigen– antibody reaction, chromogenic substrate specific to the enzyme is added.
- Ex. of **substrates** used:
 - o- phenyldiamine dihydrochloride for peroxidase
 - p-nitrophenyl phosphate for alkaline phosphatase

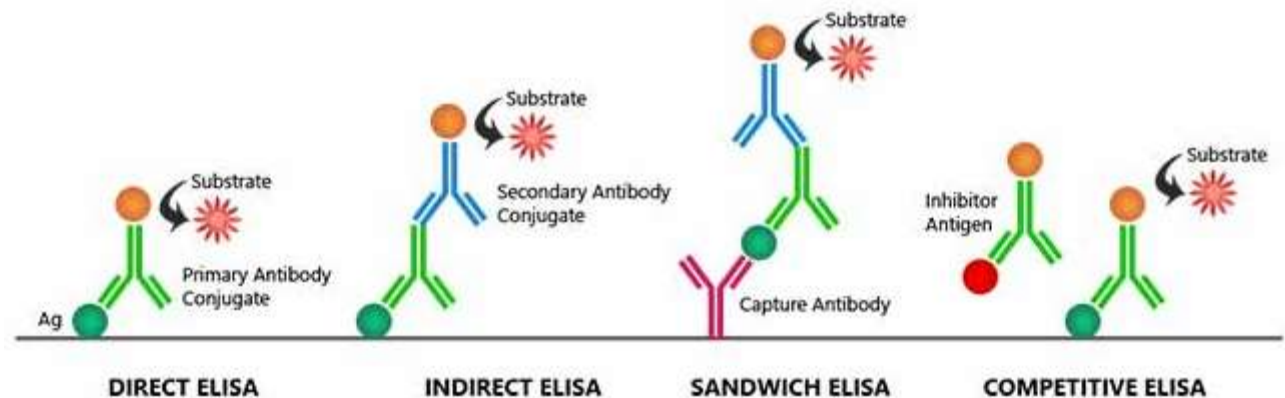
- In EIA techniques, antigen or antibody labeled with enzymes are used.
- Ex. of enzymes used in the EIA tests:
 - **Alkaline phosphatase**
 - **Horseradish peroxidase and**
 - **β -galactosidase**
- The substrate is hydrolysed by **enzyme** attached to antigen-antibody complex to give **color change**.

- **Measurement:**
- The color in reaction can be read visually or
- The reaction is detected by reading the optical density (colorimetry) using microassay plate reader i.e. ELISA reader.

- A **standard curve** based on **known concentrations of antigen** or **antibody** is prepared from which the unknown quantities are calculated.

Types of ELISA

- There are different types of ELISAs available for the detection and quantitation of either the antigen or antibodies in serum and other body fluids.
- There are four types of ELISA tests:
 - Direct ELISA
 - Indirect ELISA
 - Sandwich ELISA
 - Competitive ELISA



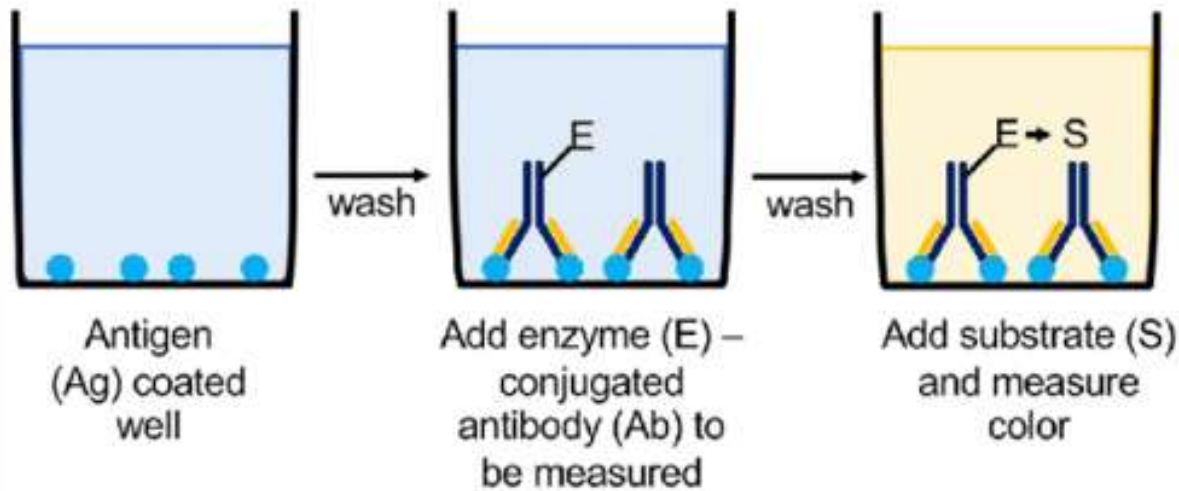
Direct ELISA

- Antigen is attached to a polystyrene plate.
- Enzyme-labeled antibody is added that can react with the antigen and a substrate that can be measured.

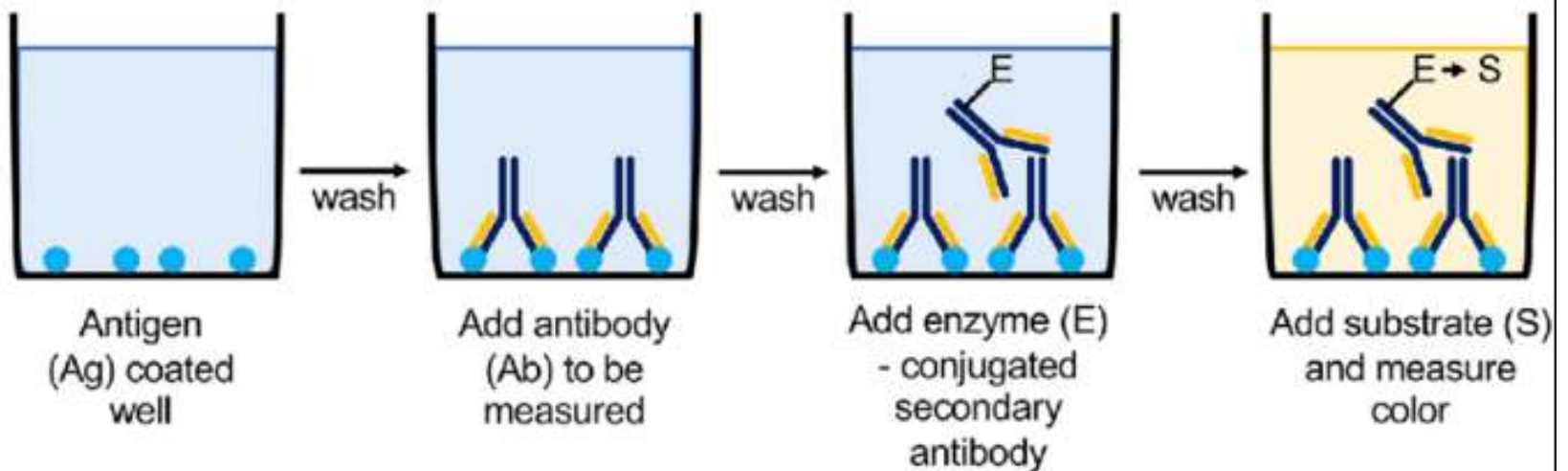
Indirect ELISA

- Antigen is attached to a polystyrene plate.
- Addition of primary antibody followed by an enzyme-labeled antibody that can react with both the primary antibody and substrate.

(a) Direct ELISA



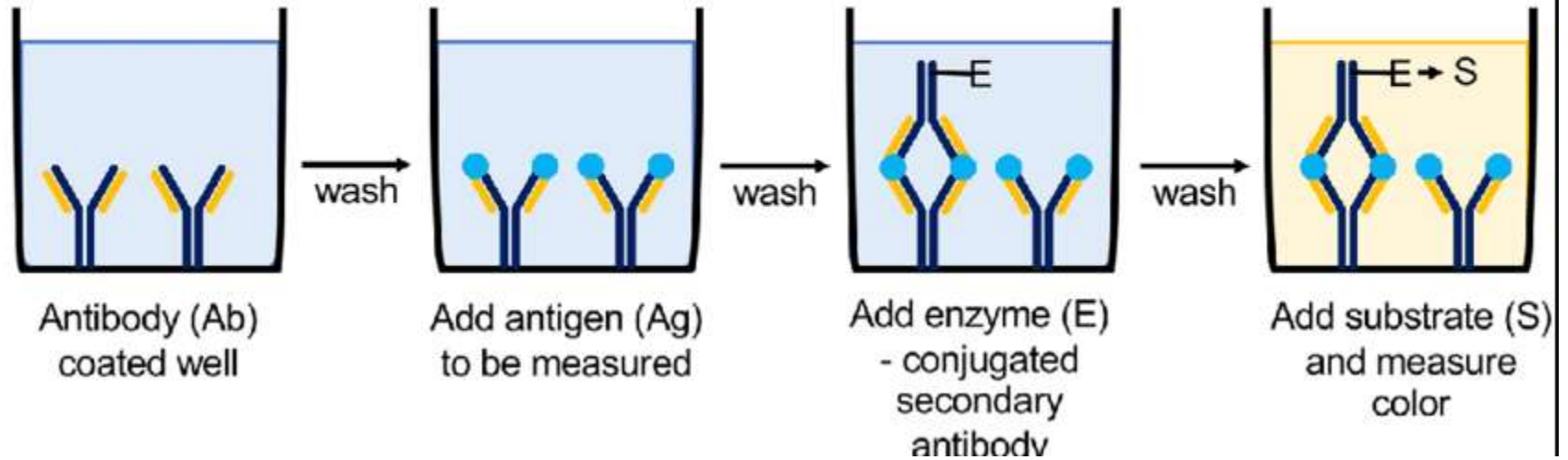
(b) Indirect ELISA



Sandwich ELISA

- A capture antibody is attached to the polystyrene plate, then antigen is added that specifically attaches or captures the antigen.
- A second antibody, also specific for the antigen but not the same as the capture antibody is added and “sandwiches” the antigen.
- This second antibody is then followed by an enzyme-labeled antibody specific for the second antibody that can react with a substrate that can be measured.

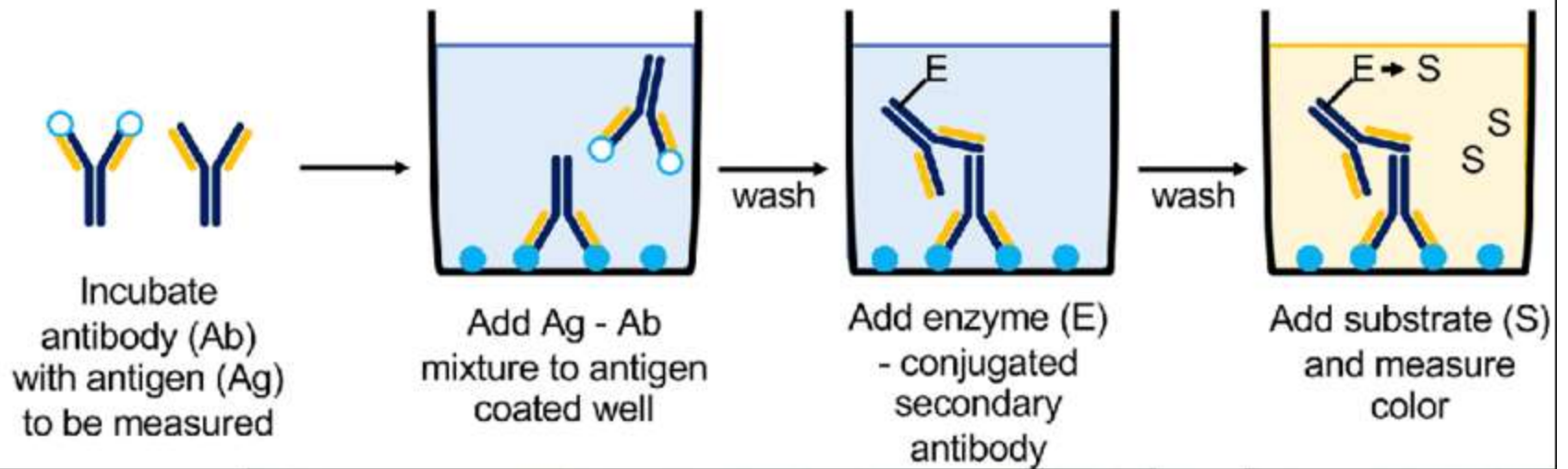
(c) Sandwich ELISA



Competitive ELISA

- This test is like the sandwich ELISA but involves the addition of competing antibodies or proteins when the second antibody is added.
- This results in a decrease in the substrate signal that is generated.
- This test is considered to give good, highly specific results.

(d) Competitive ELISA



Uses of ELISA:

- Enzyme immunoassays (EIAs) can be used for detection of either antigens or antibodies in serum and other body fluids of the patient.
- Examples of the uses of an ELISA test includes:
 - to diagnose infections such as HIV (human immunodeficiency virus)
 - and some allergic diseases like food allergies.
- ELISA tests are also known as an immunosorbent assay.