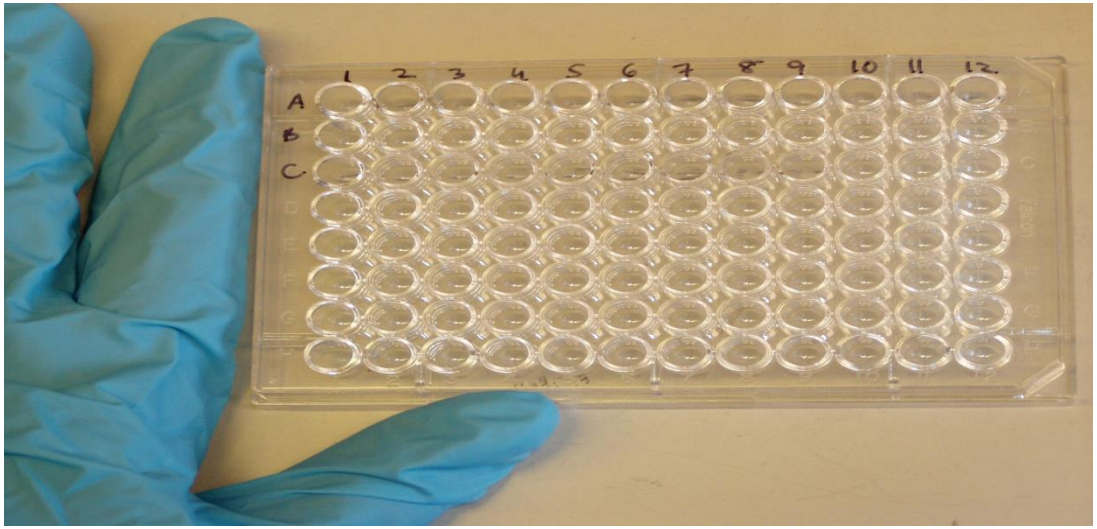
ELISA and other Techniques

(Principles and Protocols)

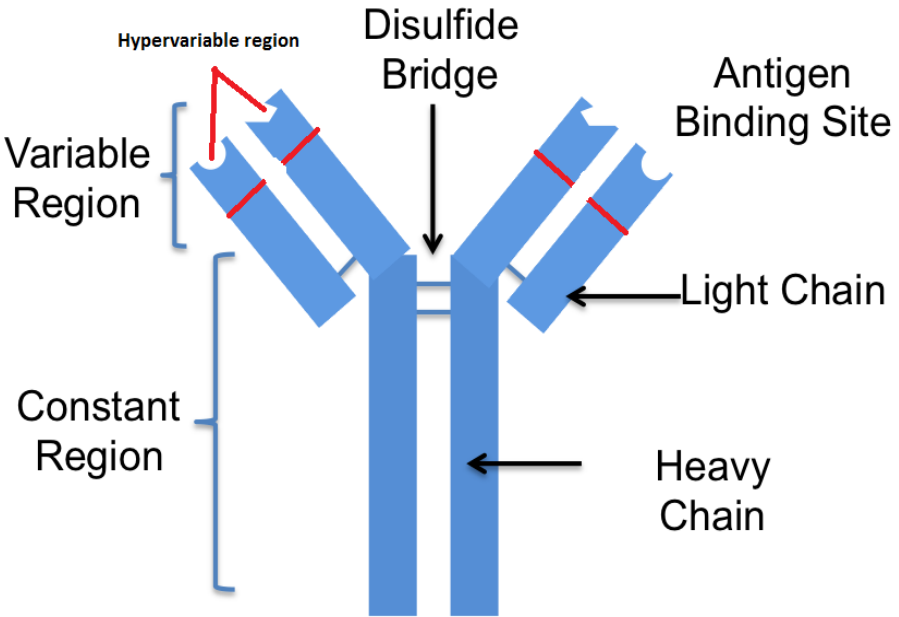
ELISA(Enzyme Linked immunosorbent Assay)

- A laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample.



Antibodies

- (also known as immunoglobulins abbreviated Ig) are gamma globulin proteins that are found in blood and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses



Antigen

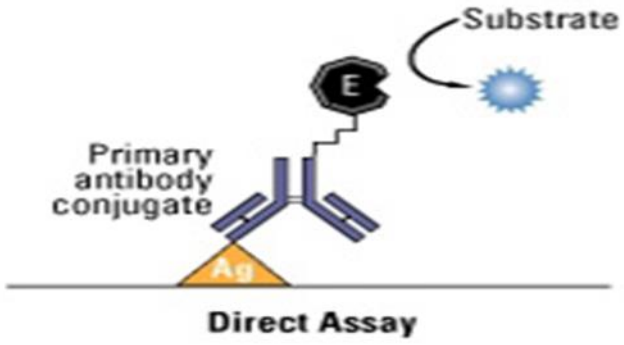
- A substance that when introduced into the body stimulates the production of an antibody.

Types of ELISA

- Direct ELISA
- In Direct ELISA
- Competitive ELISA
- Sandwich ELISA

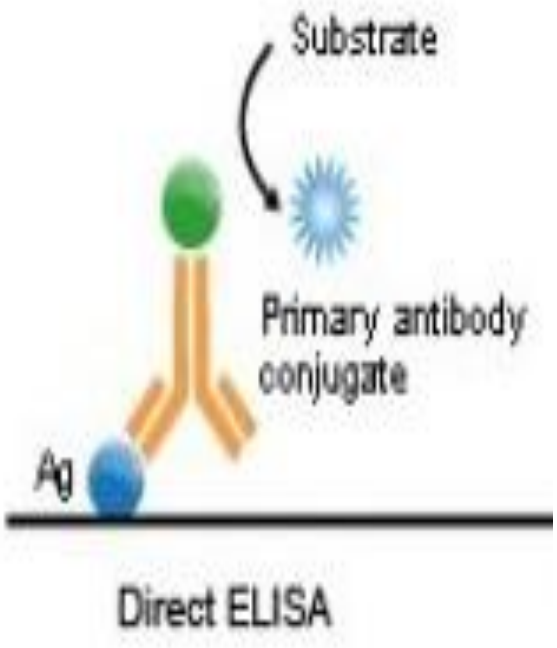
Direct ELISA

- the antigen is adsorbed to a plastic plate, then an excess of another protein (normally bovine serum albumin) is added to block all the other binding sites. While an enzyme is linked to an antibody in a separate reaction, the enzyme-antibody complex is applied to adsorb to the antigen.



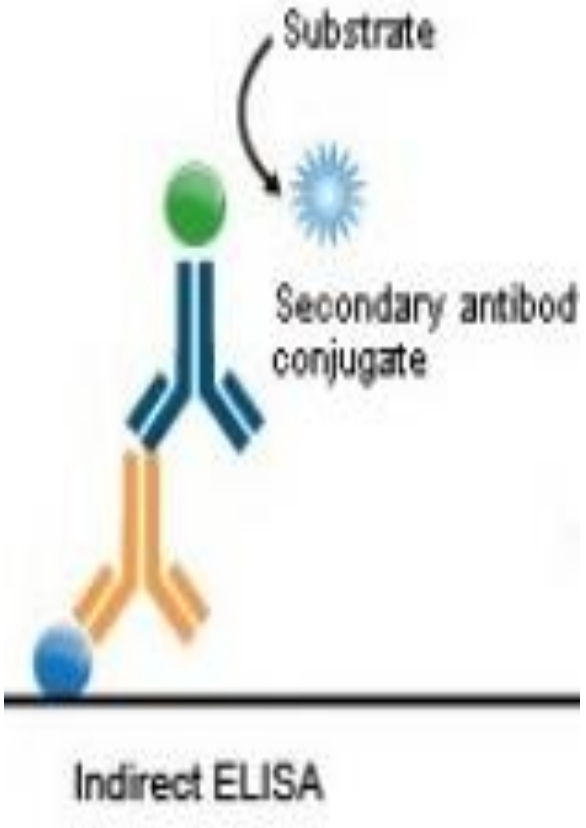
Direct ELISA

- After excess enzyme-antibody complex is washed off, enzyme-antibody bound to antigen is left. By adding in the enzyme's substrate, the enzyme is detected illustrating the signal of the antigen.



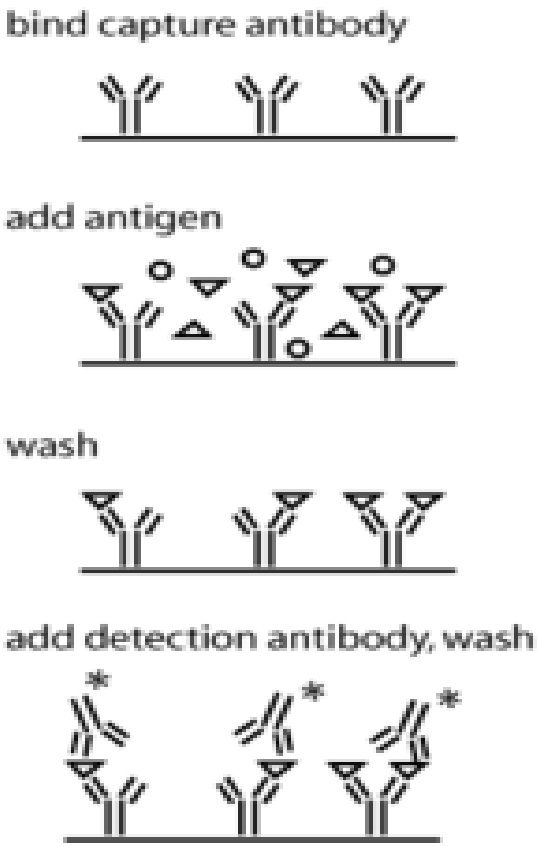
Indirect ELISA

- Antigen coated to a plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody.



Sandwich ELISA

- The sandwich ELISA quantify antigens between two layers of antibodies (i.e. capture and detection antibody). The antigen to be measured must contain at least two antigenic epitope capable of binding to antibody, since at least two antibodies act in the sandwich.



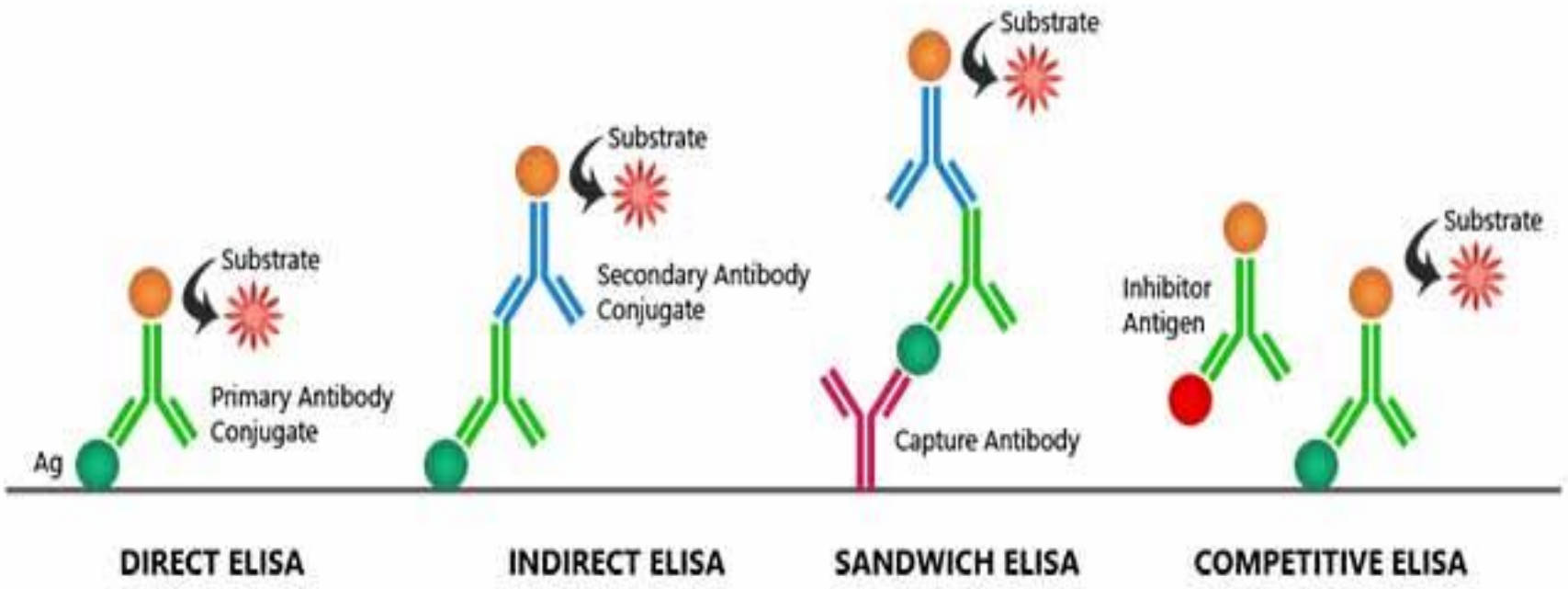
Competitive ELISA

- Coat plate with capture Ab
- Block the plate with BSA or detergant
- Mix sample with enzyme conjugate
- Add mix. To ElisA plate
- Wash ElISA plate
- Add colorless TMB substrate
- In case of quantity of the protein of interest more than enzyme conjugate **the intensity of the color will be low**

Competitive ELISA con.

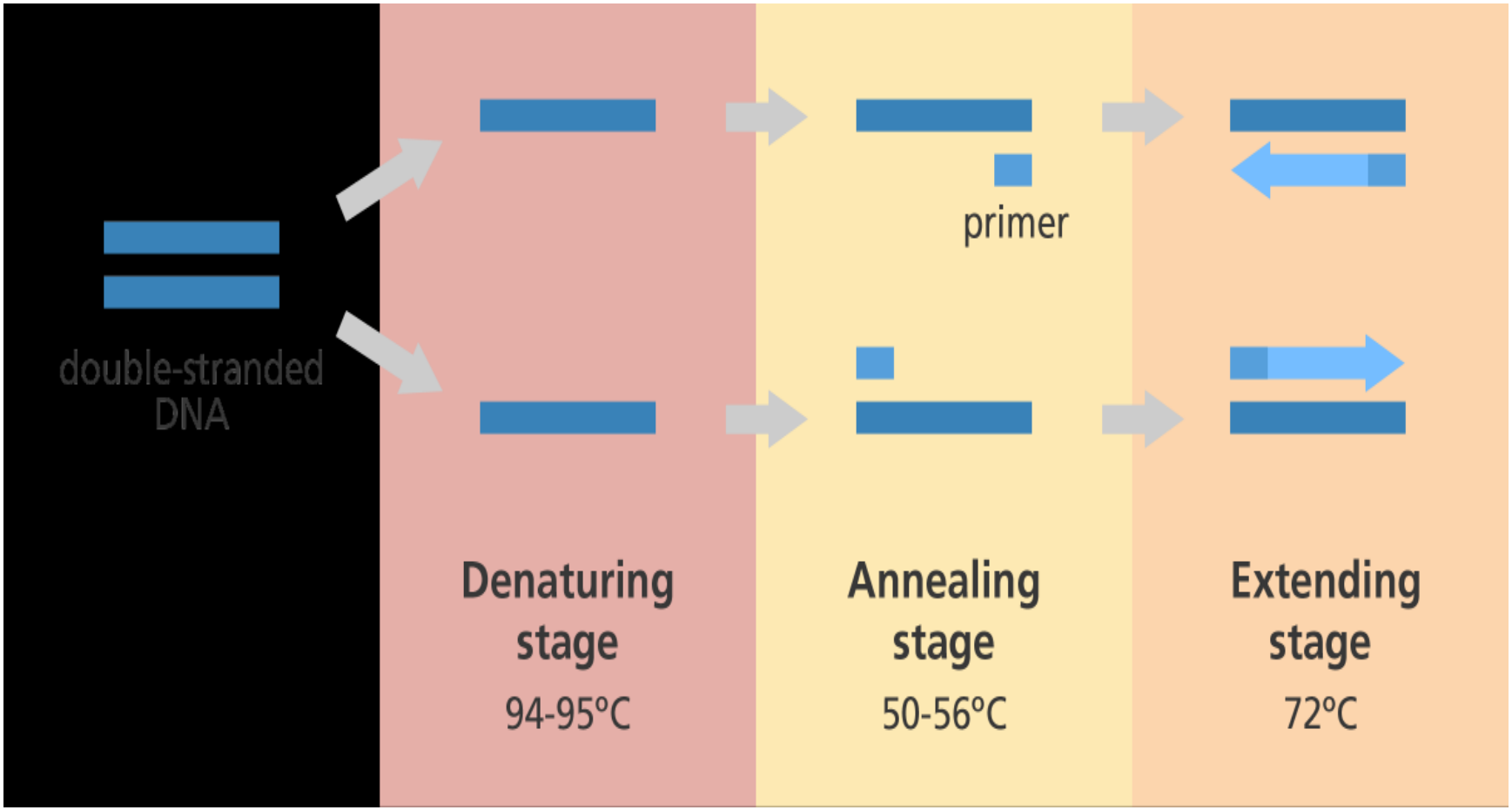
- In case of quantity of enzyme conjugate is more than quantity of protein of interest **the intensity of the color will be high**

Types of ELISA



INTRODUCTION TO: POLYMERASE CHAIN REACTION

Steps


double-stranded DNA
primer
Denaturing stage
94-95°C
Annealing stage
50-56°C
Extending stage
72°C

Steps of this Reaction

- Denaturation – when the double-stranded template DNA is heated to separate it into two single strands.
- Annealing – when the temperature is lowered to enable the DNA primers to attach to the template DNA.
- Extension – when the temperature is raised and the new strand of DNA is made by the Taq polymerase enzyme.

Applications of PCR

- Early detection of bacterial ,viral or other parasitic infection.
- To quantify titre of the virus .
- To know Genotyping of virus (virus C)

Some tests that can be done by PCR technique :

- HCV by PCR (qualitative ,quantative)
- HBV by PCR (qualitative,quantitative)
- Polycythemia


PCR &
ELISA