Advanced laboratory technique

Lab/3

Types of ELISA

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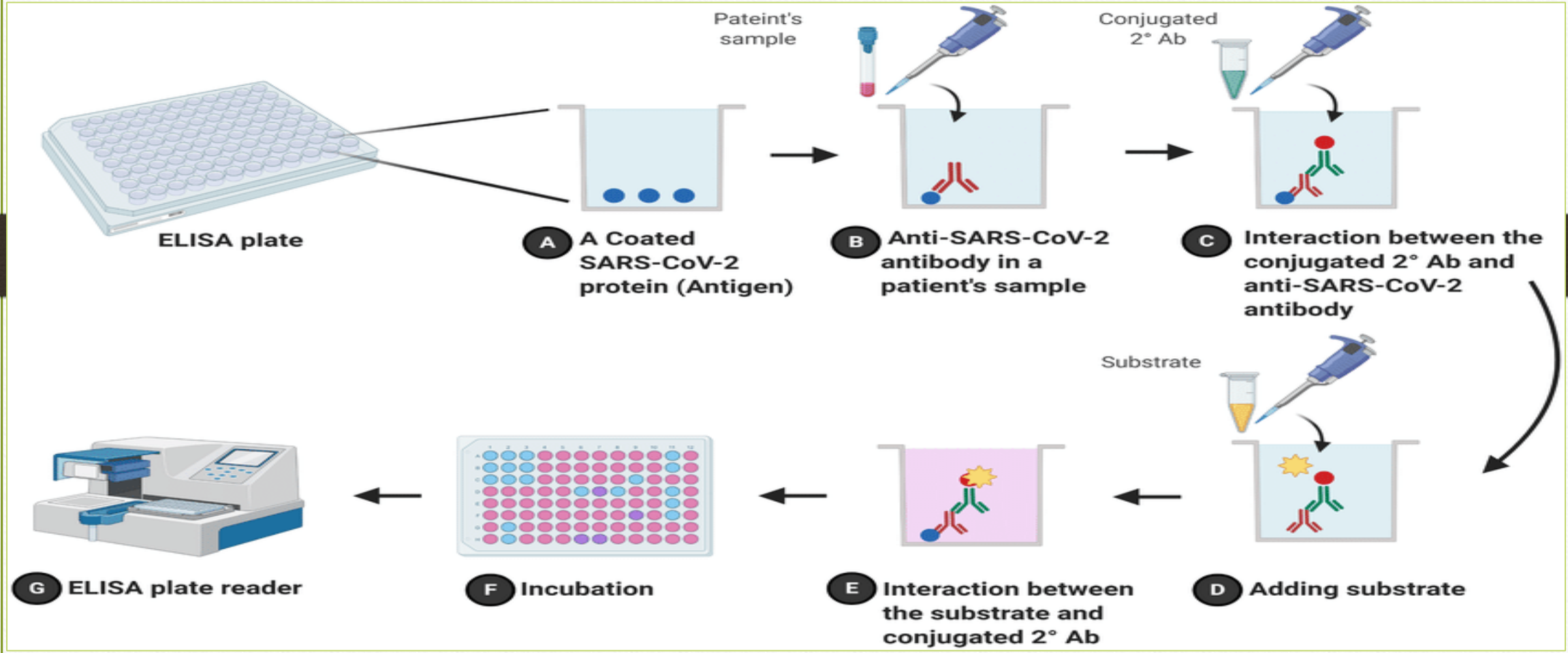
INTRODUCTION:

- ELISA(Enzyme Linked Immunosorbent Assay) is a widely used technique for detection of antigen (Ag) or antibody (Ab) .
- This immunological test is very sensitive and is used to detect and quantify substances .
- The detection of these products is accomplished by complexing antibodies and antigens to produce measurable result .

COMPONENT OF ELISA KITS:

1) Microplate: Usually a 96-well plate, pre-coated with antibodies or antigen specific to the target.
2) Conjugated detection antibody: These antibodies bind to target and are often conjugated to an enzyme, common enzymes include horseradish peroxidase (HRP) or alkaline phosphatase (AP).
3) Substrate: A chemical that reacts with the enzyme to produce a detectable signal, usually a color change. For example, TMB (Tetramethylbenzidine) .
4) Standards and controls: These are known concentrations of the target antigen used to create a standard curve and validate the assay.
5) Wash buffer: Used to wash away unbound substances between steps.
6) Stop solution: Stops the enzyme reaction to fix the color change, allowing for accurate measurement.

PRINCIPLE OF ELISA:

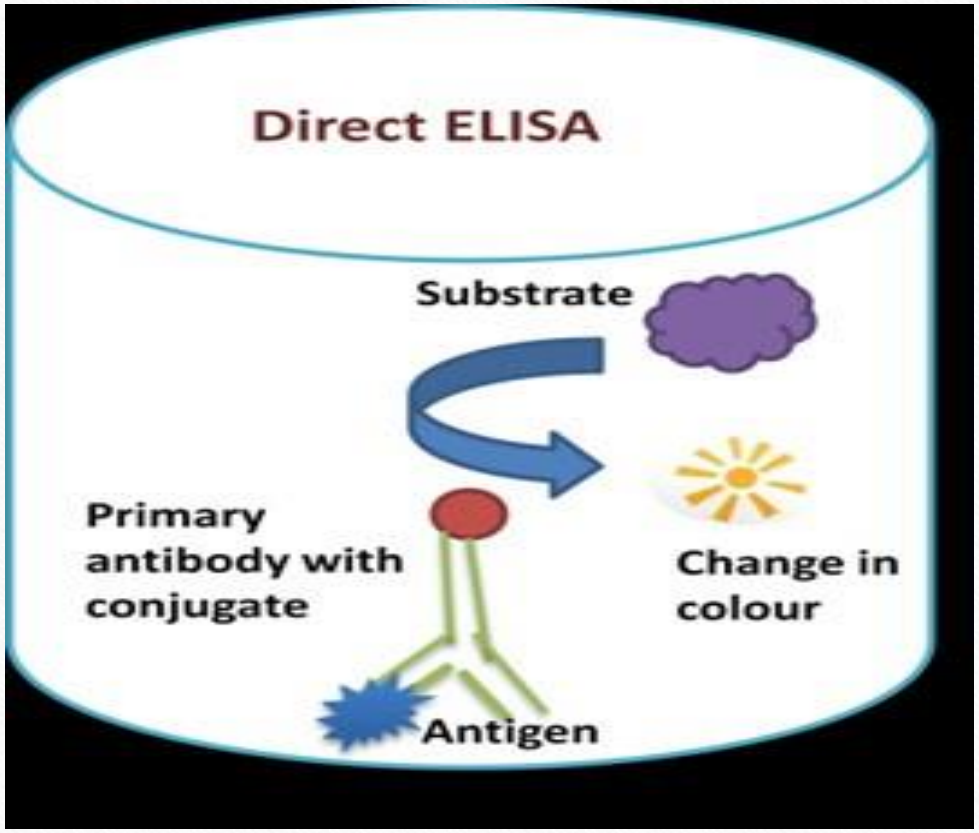


TYPES OF ELISA:

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

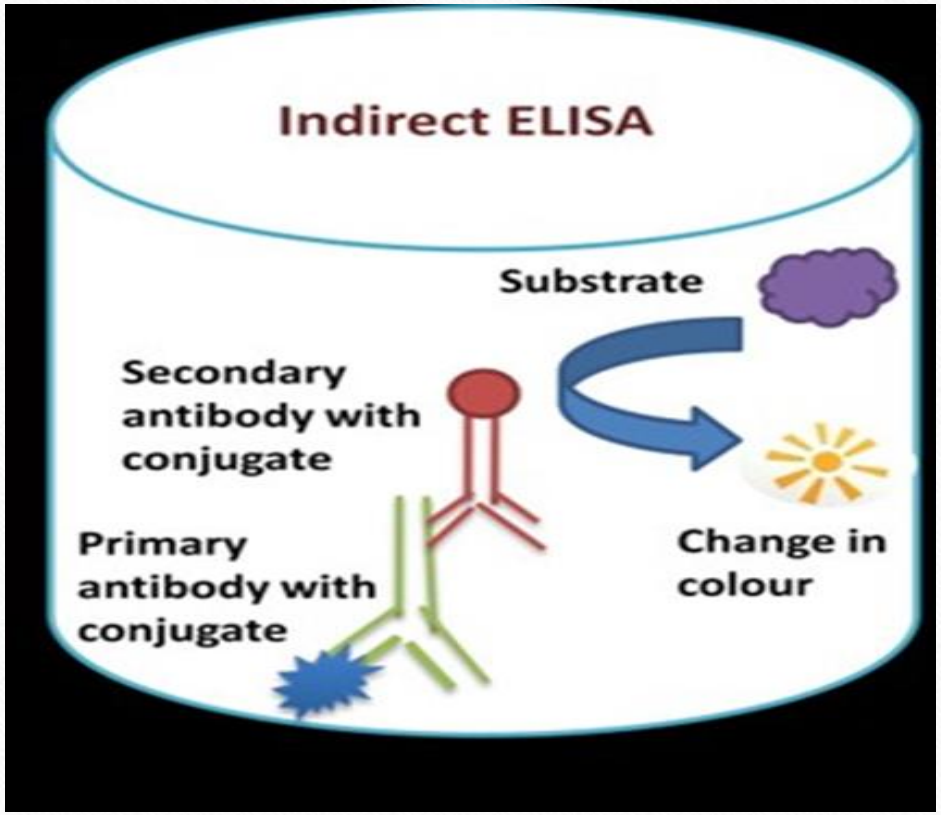
Direct ELISA:

Direct ELISA: In this type, the antigen is directly immobilized on the plate, and a labeled antibody specific to the antigen is used for detection. This method is straightforward but can be less sensitive due to the lack of signal amplification.



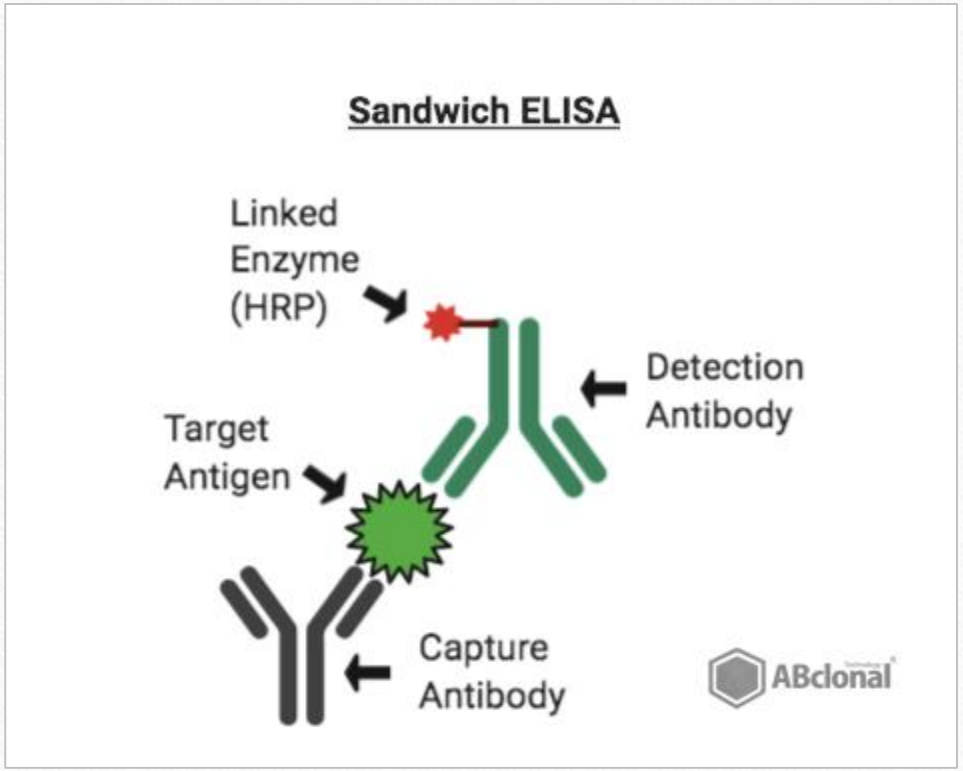
Indirect ELISA:

Indirect ELISA: the antigen is also immobilized on the plate, but detection involves a two-step process. A primary antibody binds to the antigen, followed by a labeled secondary antibody that binds to the primary antibody. This method is more sensitive than direct ELISA due to signal amplification.



Sandwich ELISA:

Sandwich ELISA: This is the most commonly used format. It requires two antibodies specific to different epitopes of the antigen. One antibody (capture antibody) is used to coat the plate and capture the antigen, while the other (detection antibody) is used to detect the antigen. This method is highly specific and sensitive.



Competitive ELISA:

Competitive ELISA: In this type, the sample antigen competes with a labeled antigen for binding to a limited amount of antibody. The amount of antigen in the sample is inversely proportional to the signal detected. This method is useful for detecting small molecules and when the antigen is in low concentration.

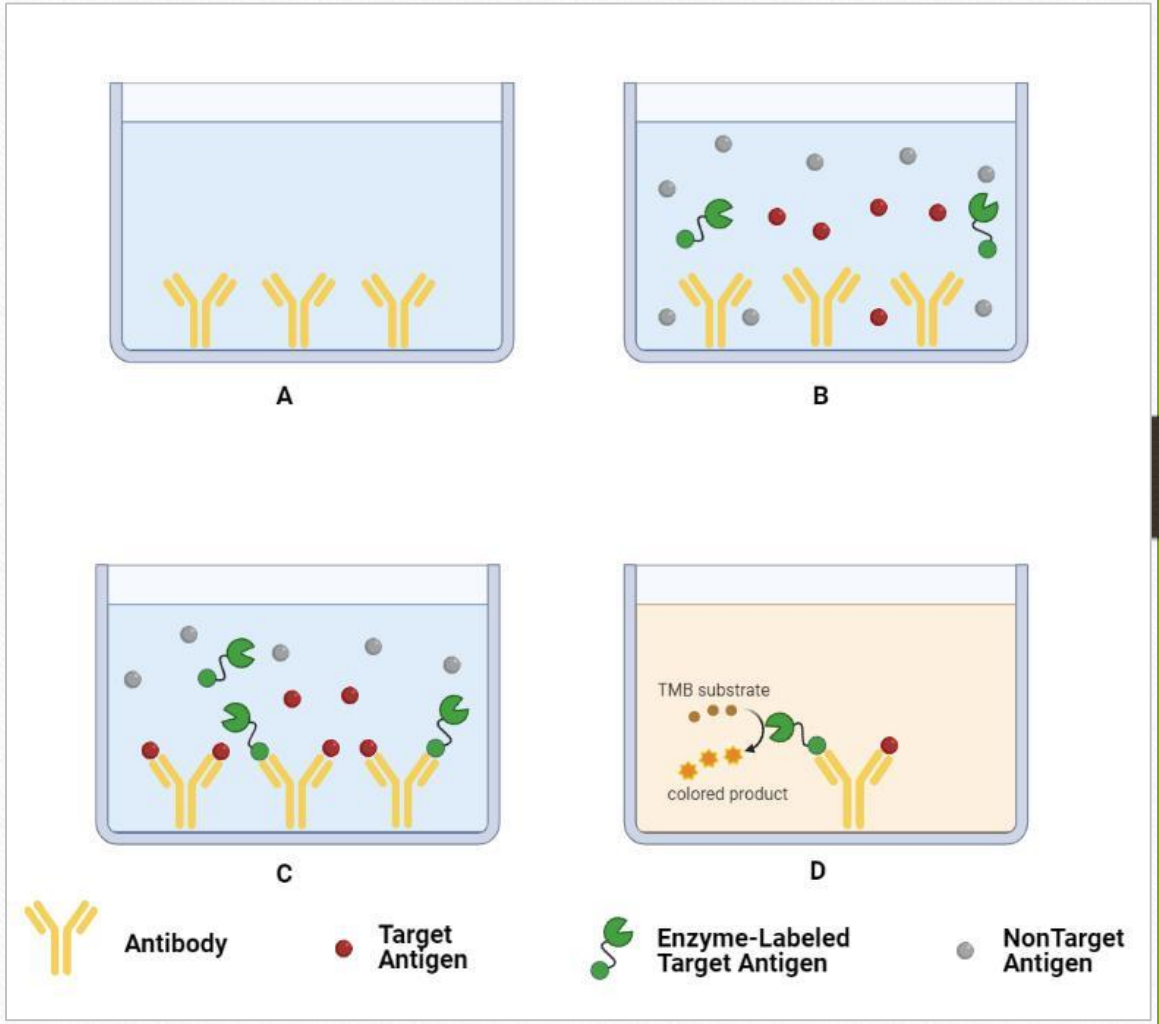



Plate
Reader


ROMER
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Thank you