

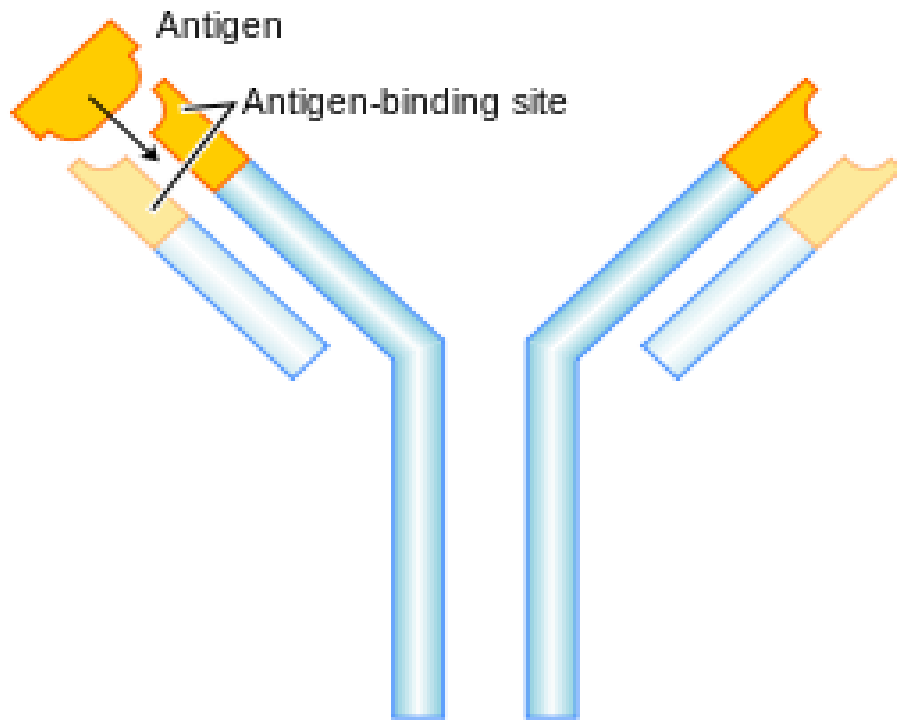
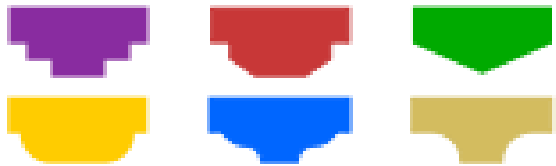


ENZYME-LINKED IMMUNOSORBENT ASSAY [ELISA]

Immunoassay:

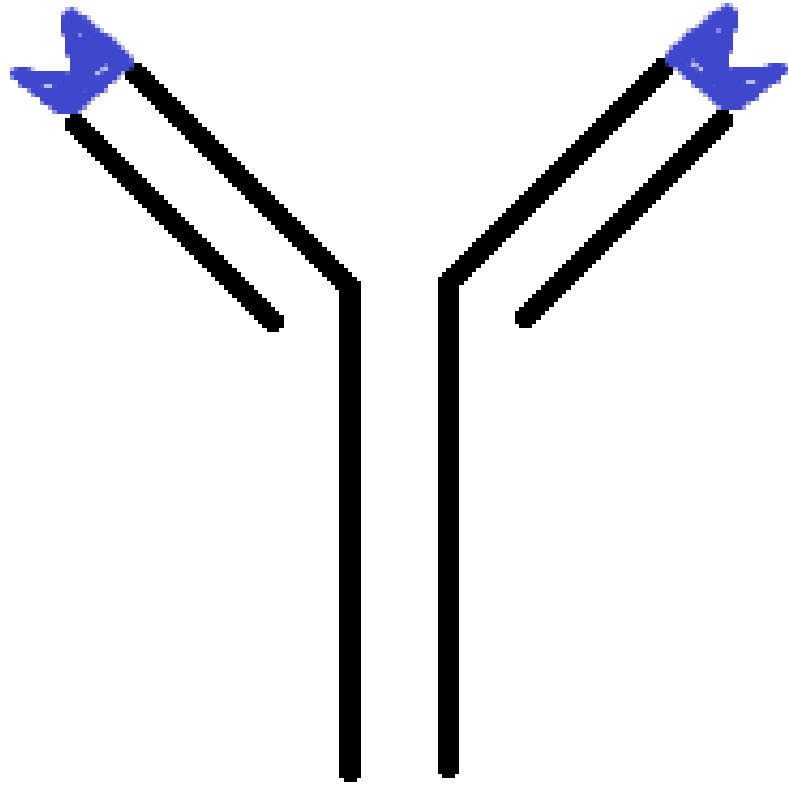
- What is antigen (Ag) ?
- What is antibody (Ab) ?
- Immunoassay ?
- Specificity ?

Antigens

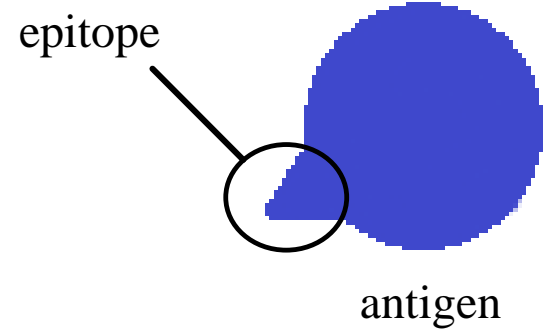


Antibody

Each antibody recognize specific antigen

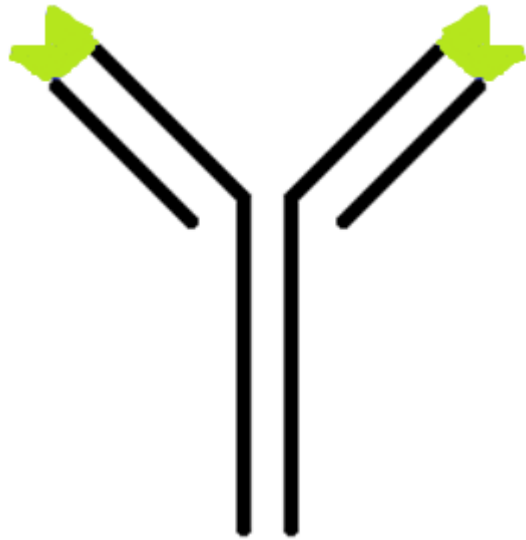


antibody



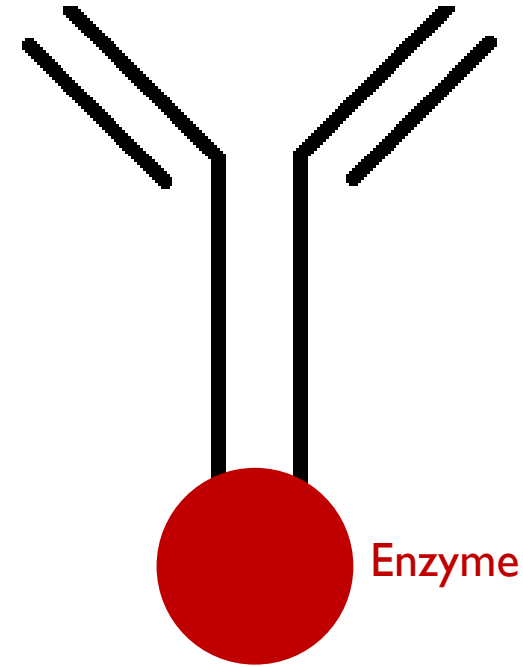
epitope

antigen



Primary antibody

“antibody specified to specific antigen”



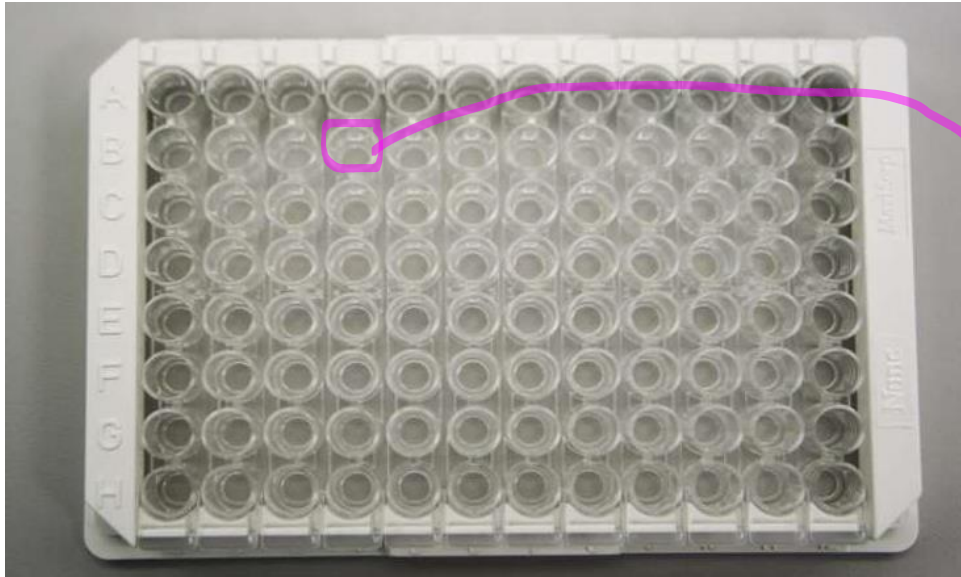
Secondary antibody

“antibody specified to Primary antibody”


ELISA:

- Enzyme-linked immunosorbent assay.
 - Is a biochemical plate-based assay technique designed for **detecting and quantifying** substances such as peptides, proteins, antibodies and hormones.
- **In qualitative ELISA:** + OR -
- **In quantitative ELISA:** The optical density or florescent units of the sample is interpolated into a standard curve.
- Application ?
 - Autoimmune disease?

96-well (or 384-well) polystyrene microtiter plates



← **microtitre plate**
solid support used
to immobilized
antigen or antibody
of interest.



Adding the sample
and incubate for 1
or 2 hr.

← **microtitre plate**
solid support used
to immobilized
antigen or antibody
of interest.

Basic Principle:

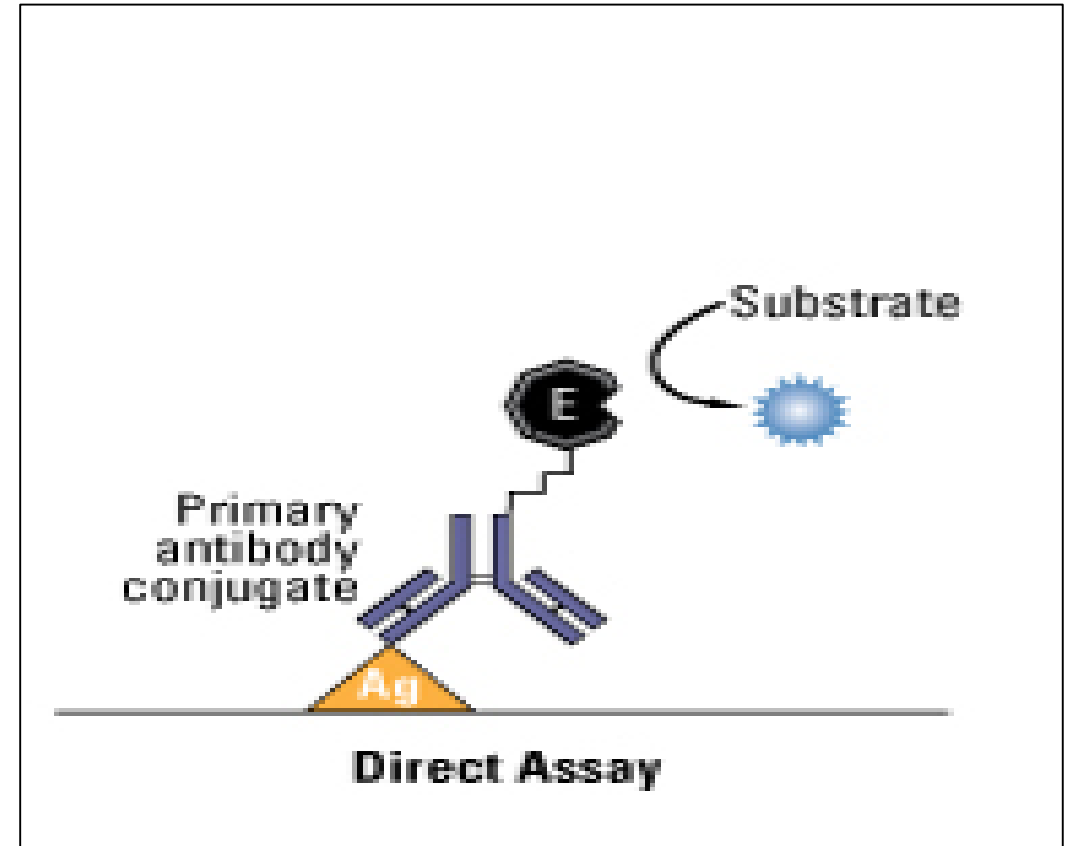
- To detect a specific antibody- antigen reaction by assessing the **conjugated enzyme activity**.
- The enzyme convert a colourless substrate to a measurable **coloured product**, indicating the presence of the antibody - antigen [Ab-Ag] binding.
- The detection enzyme can be linked **directly to the primary antibody** or introduced through a **secondary antibody** that recognizes the primary antibody.
- The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISA Format:

- Direct ELISA.
- Indirect ELISA.
- Sandwich ELISA.
- Competitive ELISA.

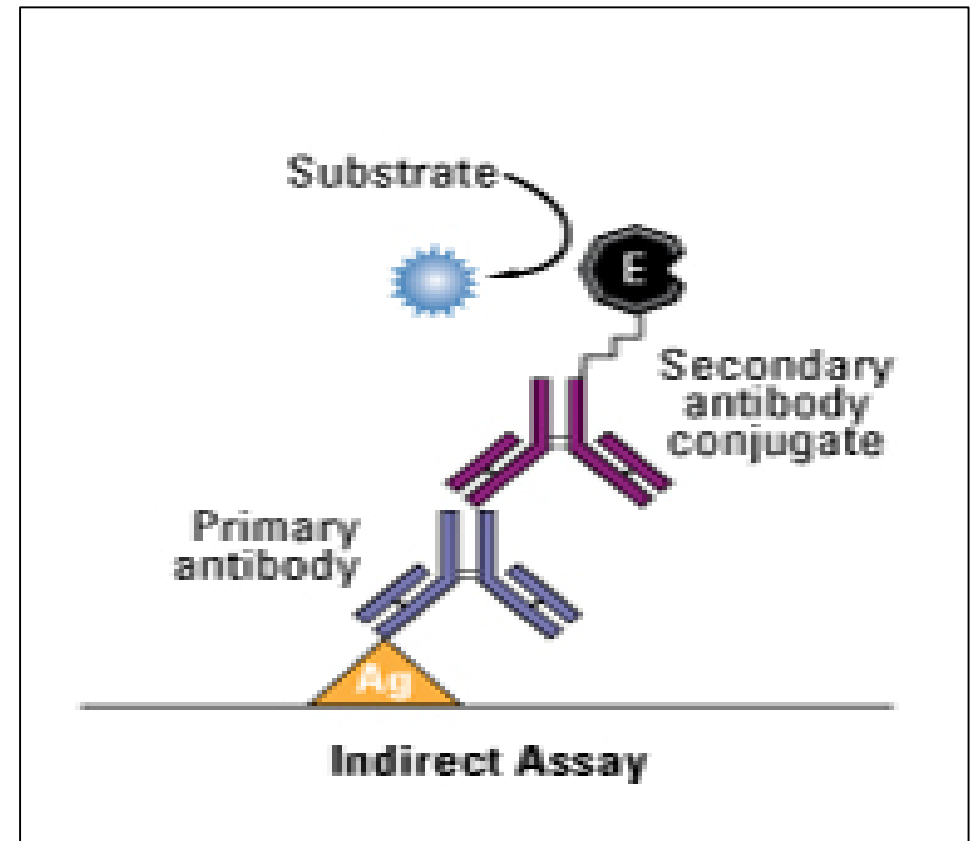
Direct ELISA:

- This type considered the simplest type of ELISA.
- It is used to detect the presence and the concentration of specific antigen in the sample.
- Why this format called "direct ELISA" ?



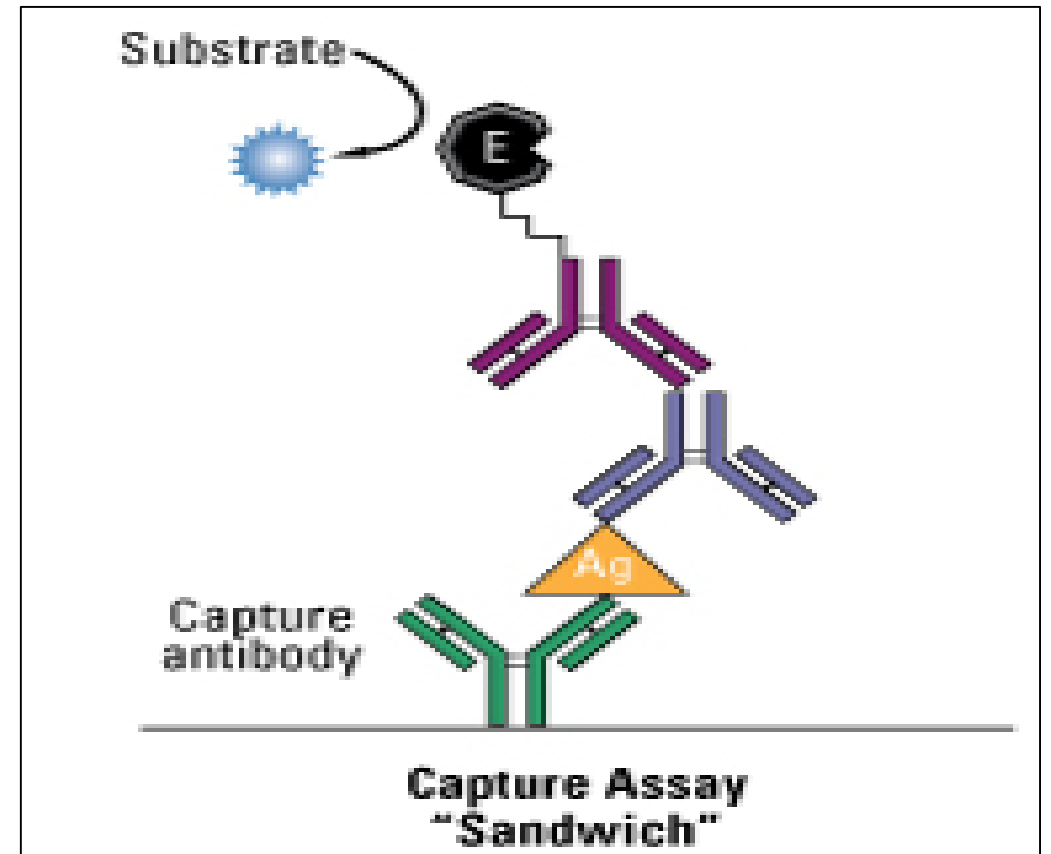
Indirect ELISA:

- Is used to detect the presence and the concentration of specific antigen or antibody.
- Why this format called “Indirect ELISA” ?

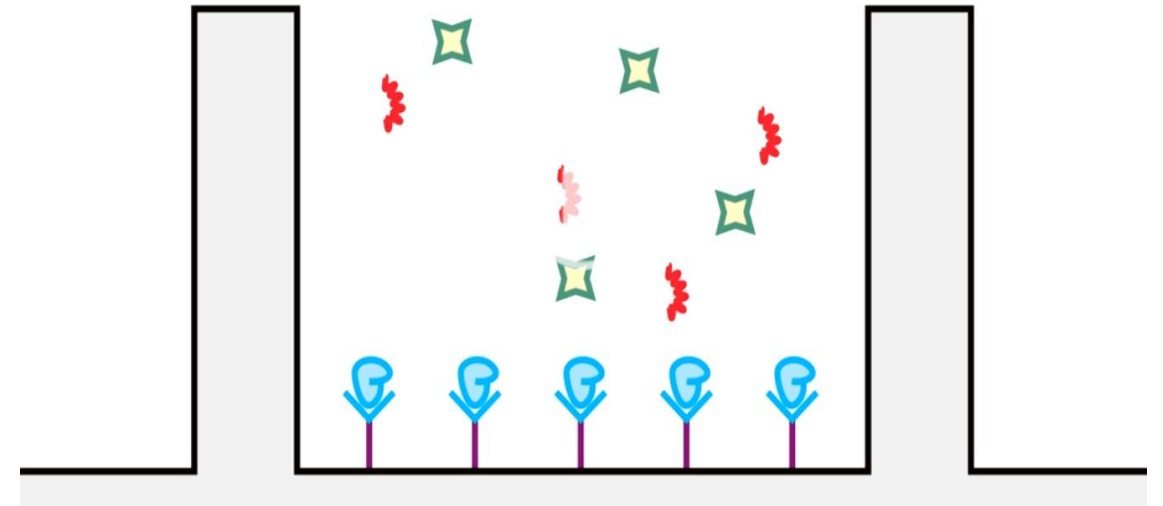


Sandwich ELISA:

- The most powerful ELISA assay format is the sandwich assay.
- This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody.
- It is used to detect the presence and the concentration of specific antigen in the sample.

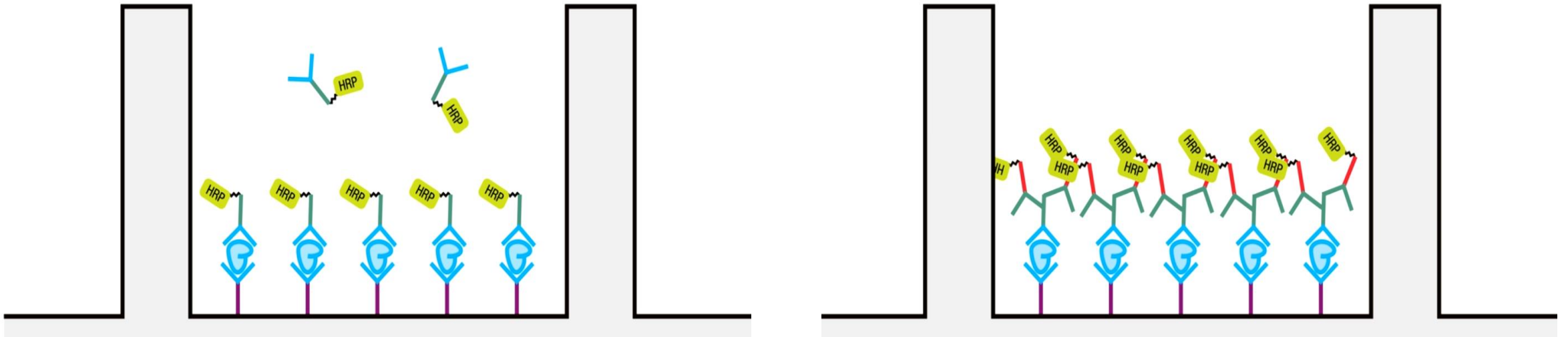


Direct or Indirect Capture?



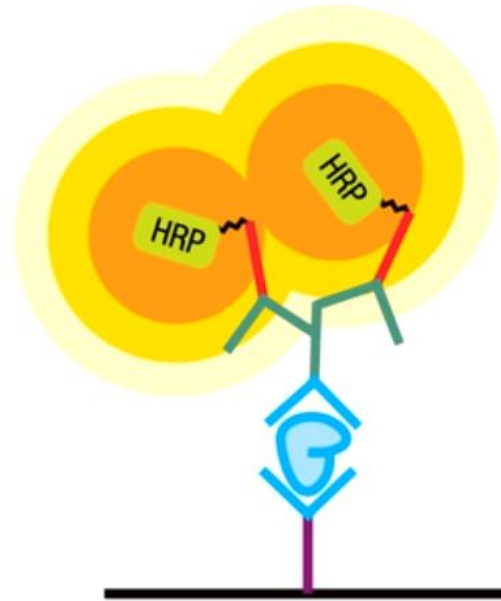
Antigen of interest

Direct or Indirect detection?

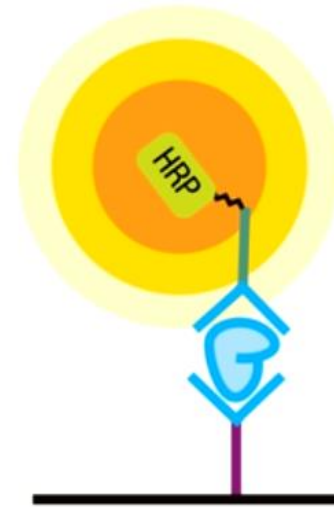


Antigen of interest

Signal amplification



Indirect detection



Direct detection

Competitive ELISA:

- Is a strategy that is commonly used when the antigen is small and has only one epitope, or antibody binding site.
- It measures the amount of antigen in a sample.
- One variation of this method consists of **labelling purified antigen instead of the antibody**.

