ADVANCED LABORATORY TECHNIQUES

ELISA

جامعـــــة ســـــاوة الاهلية — Sawa University

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المرحلـــــــــة الثالثة — third Stage .

محاضرة رقم 4

Lecture No. 4

الجانب النظري

Theoretical

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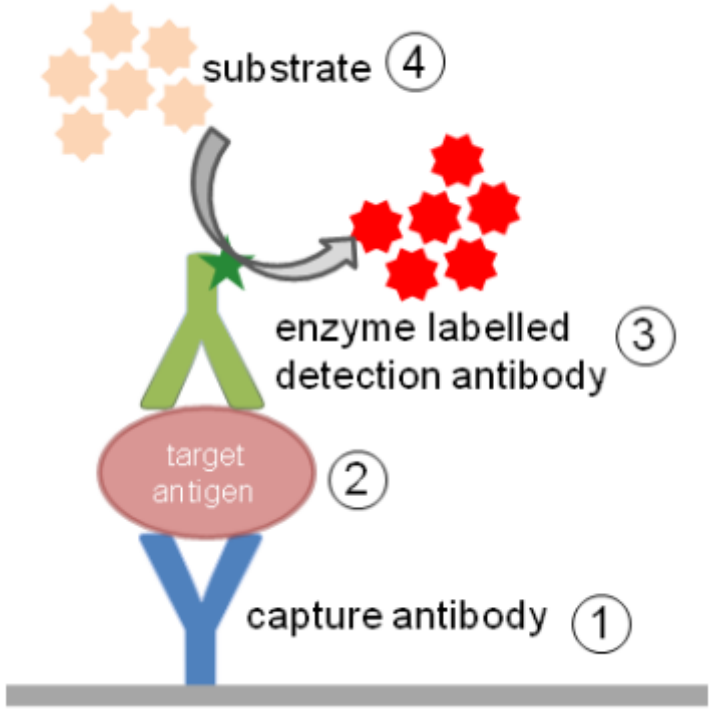
(INTRODUCTION)

- **ENzyme-linked immunosorbent assay (ELISA):** is an enzyme immunoassay used to detect the presence of antigens or antibodies in biological samples.
- It is widely applied in the diagnosis of infectious diseases (e.G., Hepatitis, HIV), monitoring immune responses, and detecting biomarkers. Advantages over radioimmunoassay include safety (no radiation), longer shelf-life of reagents, and simpler equipment.

Principle of ELISA:

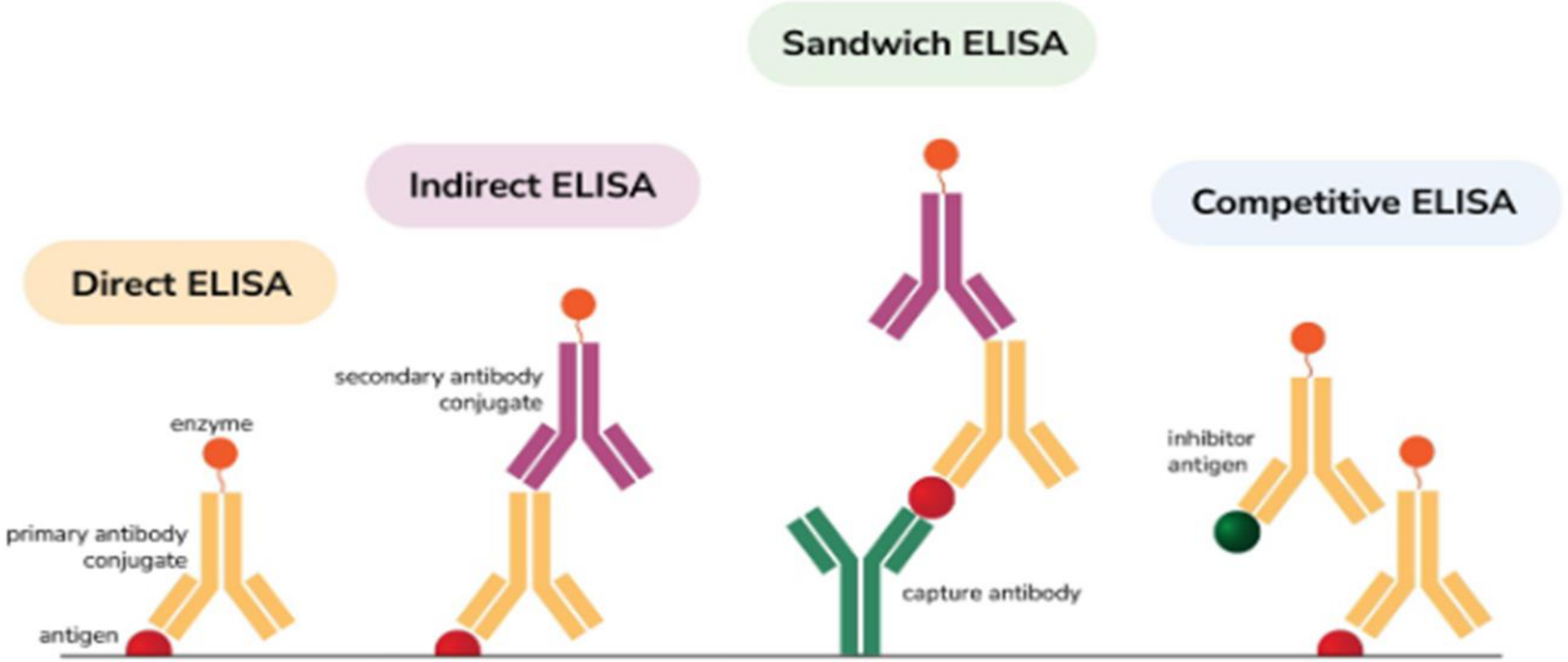
Based on **specific antigen-antibody interaction**. Uses an **enzyme-conjugated antibody (or antigen) as a reporter**. Addition of **substrate leads to a colorimetric reaction measured visually or with a spectrophotometer.**



Types of ELISA :

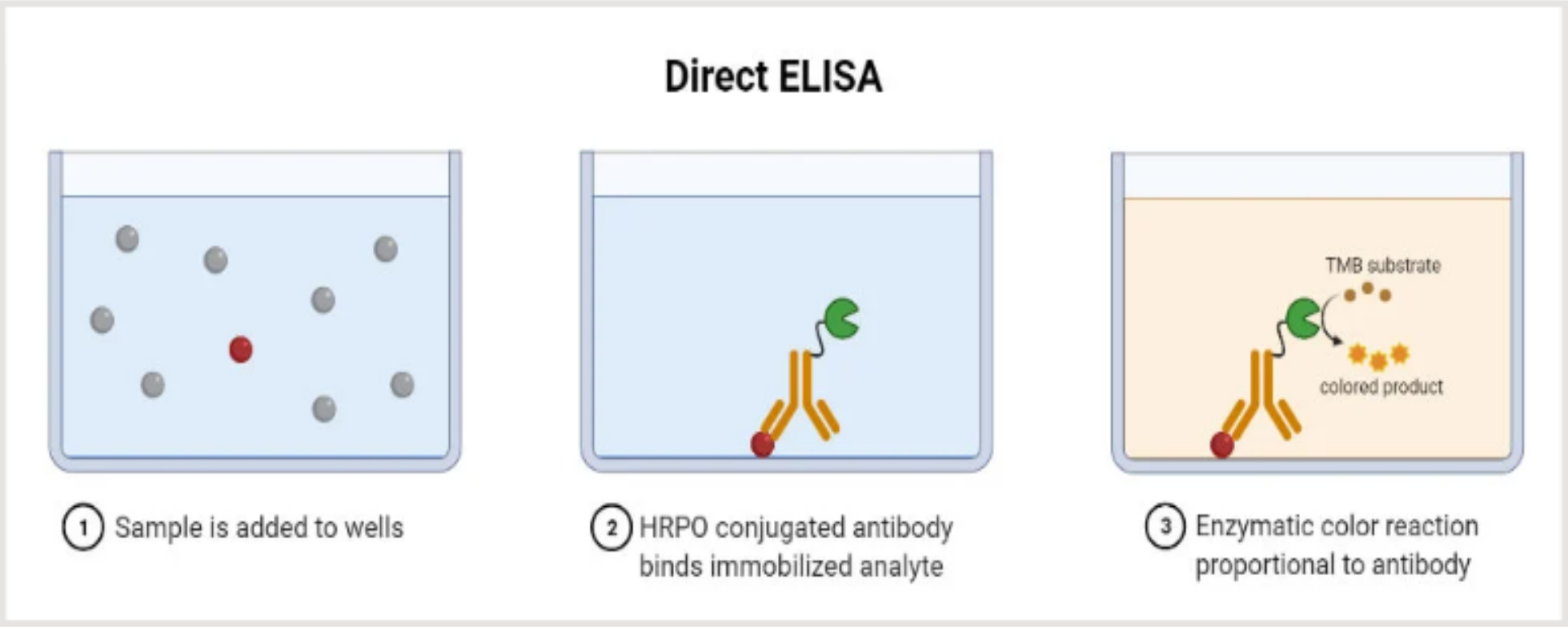
- direct ELISA – antigen fixed, detected by enzyme-labeled antibody.
- Indirect ELISA – antigen fixed, detected by primary antibody + enzyme-labeled secondary antibody.
- Sandwich ELISA – antibody fixed, captures antigen, detected by a second labeled antibody.
- Competitive ELISA – labeled and unlabeled antigens compete for antibody binding

Types of ELISA



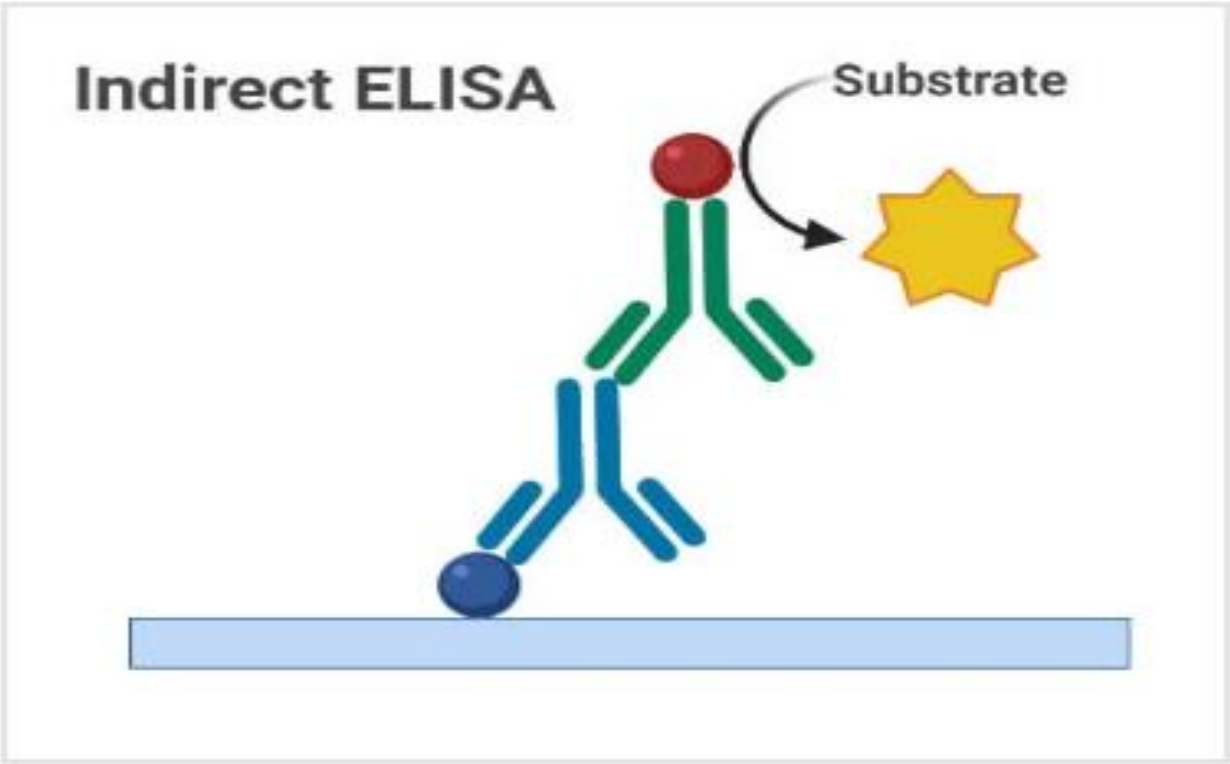
1. Direct ELISA

It is the simplest and quickest of all other types of ELISA. A single enzyme-linked antibody is used which directly interacts with the antigen present in the sample.



2. Indirect ELISA

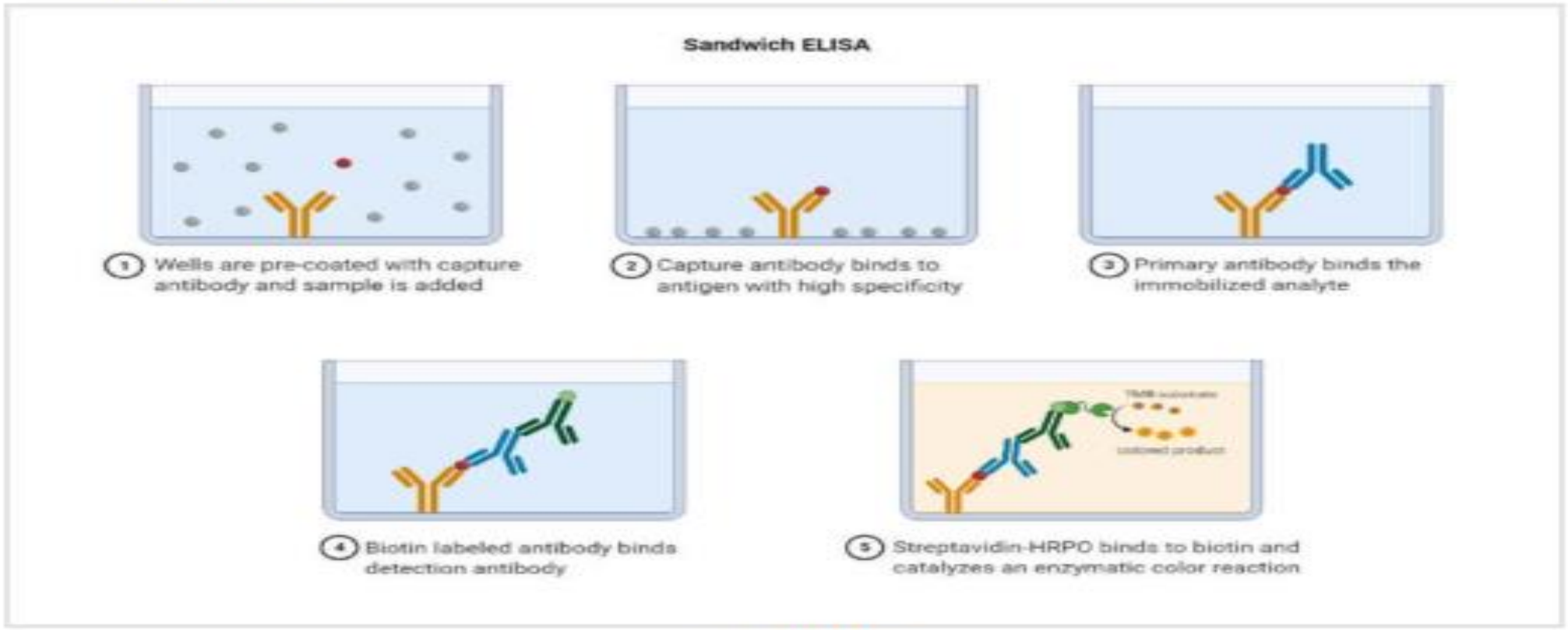
- Developed in 1978.
- It is the most popular type of ELISA in use.
- Antibody detection is carried out in indirect ELISA. A secondary antibody linked with the enzyme is used.



Indirect ELISA

3. Sandwich ELISA

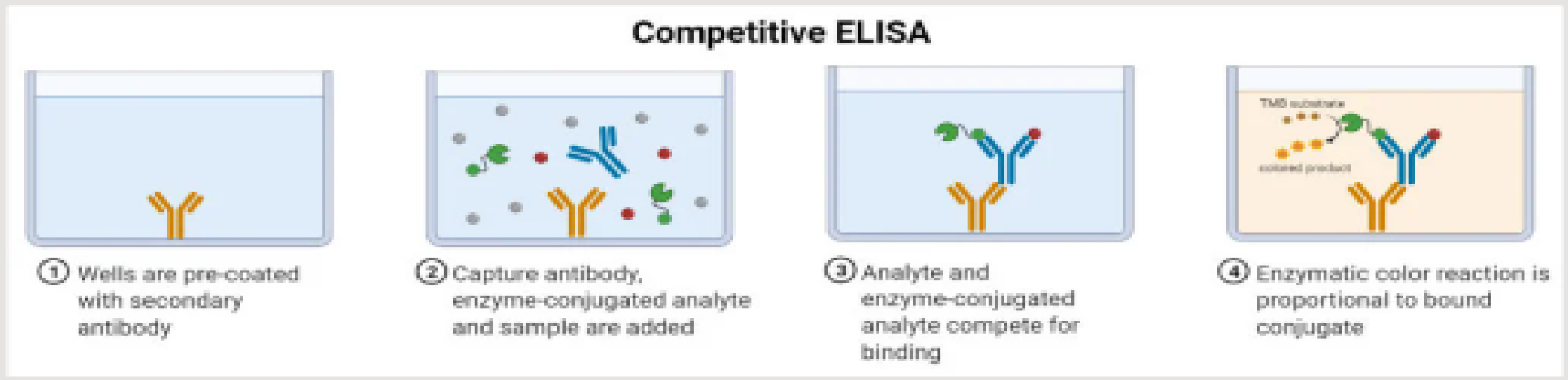
- Developed in 1977.
- Detection of antigens.
- Two different antibodies i.e. capture antibodies(for attachment sample antigens) and enzyme-linked secondary antibodies are required.
- The antigen of interest first binds with the capture antibodies and then the secondary antibodies bind with the epitope antigen forming a sandwich-like structure with antigen in between antibodies.



Sandwich ELISA

4. Competitive ELISA

- Developed in 1976.
- Detection of antibodies.
- The basic concept of this type is that there occurs competition of binding between the sample antibodies(if present) and enzyme-linked secondary antibodies to the antigens. If the sample contains specific antibodies of interest, they will bind to the antigens and won't allow enzyme-linked antibodies to bind with antigens which are then washed away. Hence after the addition of substrate, there won't be a color change indicating a positive test and vice-versa.



Competitive ELISA

ELISA Requirements

- Coated plates (Microtitre plates): Commonly used ones are 96 well polystyrene plates. Coated with antigens or antibodies at the bottom of the well.
- Sample diluents: To dilute the sample before application in some cases of ELISA test.
- Wash Buffers: Help to wash away the unrequired contaminants and unbound antigens or antibodies. Eg. Triphosphate buffer (ph 7.40) and detergents such as Tween-20.
- Enzyme-linked Antibodies: For this, most common enzymes used are :
  - AP(Alkaline Phosphatase)
  - HRP(Horseradish Peroxidase)
- Substrates: Specific chromogenic substrates are used for the respective enzymes used. Commonly used substrates are o-phenylene diamine for HRP enzyme and p-nitrophenyl phosphate for AP enzyme.
- Stop solution: It stops the enzyme and substrate reaction. It can be acids such as sulphuric acid.

ELISA Applications:

- ✓ It is one of the most sensitive and effective methods for the detection of different viral, bacterial, and fungal infections.
- ✓ Screening test for HIV infection.
- ✓ Different EISA test kits are available for the detection of dengue fever, TB, and Hepatitis B infections.
- ✓ Pregnancy test kits based on ELISA are also available.
- ✓ Qualitative and quantitative estimation of various proteins, hormones, toxins, etc.
- ✓ Also used in the detection of different food allergens.

Advantages & limitations:

- **advantages:**

- ✓ high sensitivity and specificity.
- ✓ Relatively easy, inexpensive, and safe.
- ✓ Can be quantitative or qualitative.

- **Limitations:**

- requires careful standardization.
- Risk of false positives (needs confirmation e.G., Western blot).
- Time-consuming compared to rapid tests.

QUIZ

H.W:

If two patients have the same amount of antigen in their blood, why might one patient's ELISA test show a stronger color reaction than the other?

Any questions?