ELISA

- Enzyme- linked immunosorbent assay (ELISA) is a common laboratory technique which is used to measure the concentration of an analyte usually **antigens of infectious agent or antibodies against them in bodily fluids.**

- In this technique, **the antigen or antibody** from the sample tested is **immobilised from the liquid phase on to solid phase**, typically **wells of microtitre plate**.

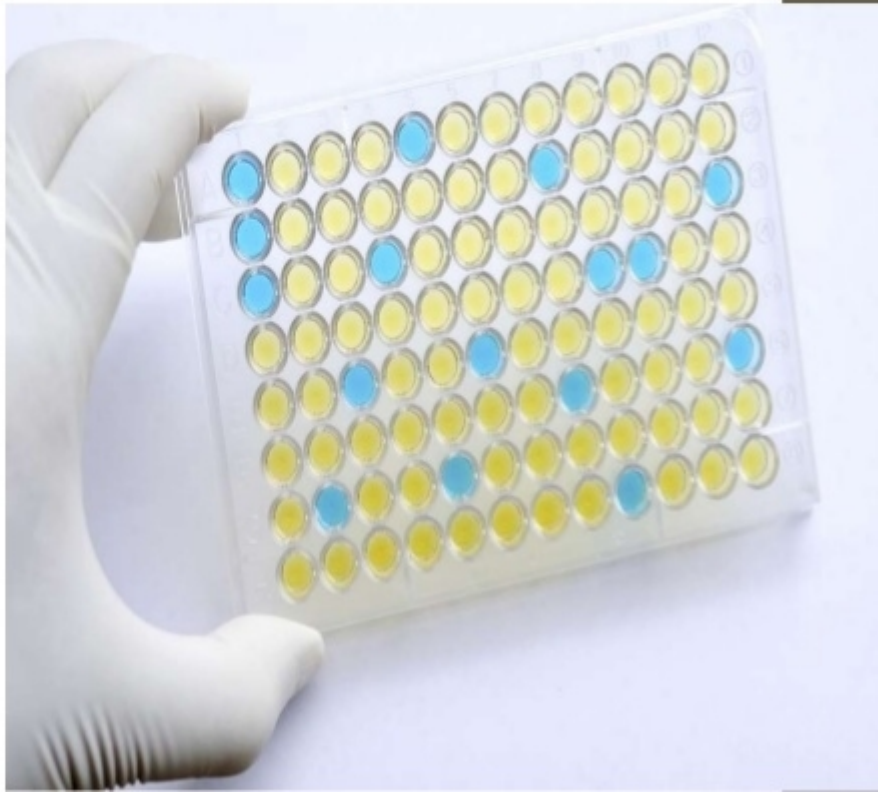
Principle of ELISA

- Based on basic immunological response i.e. formation of **antibody-antigen complex** and its detection by the reaction with another antibody, which is **labelled with a reporter enzyme** producing a signal in the form of **change in color** when specific substrate is added.

Equipments:

1) Microwell Plate:

Flat bottom polystyrene plate, contains 8 x 12 wells holding 350 µL each.



Equipments:

2) Multipipette :

An 8-channel 100 µL pipette is a good help for even small-scale work.

Equipments:

4) Microplate washer:

- These are very efficient with unusually low carry-over contamination.

Reagent	composition
Coating Buffer	0.01 M Phosphate Buffer +0.1M NaCL
Diluting /Washing Buffer	Phosphate Buffer + NaCL +0.1% Tween 20
Blocking Buffer	BSA
Enzyme	HRP,ALP
Chromogenic Substrate	TMB, PNPP
Stop Solution	0.5 M Sulfuric Acid

Double Antibody Sandwich ELISA

- DAS ELISA also called **direct ELISA** is probably the most widely used **immunochemical technique** in diagnostics.
- The principle is that **antigen is immobilised** on a solid phase by a **primary coating antibody** and **detected with a second antibody that has been labelled with a marker enzyme**
- The antigen creates a **bridge between the two antibodies** and the presence of the enzyme causes a **color change in the chromogenic substrate**

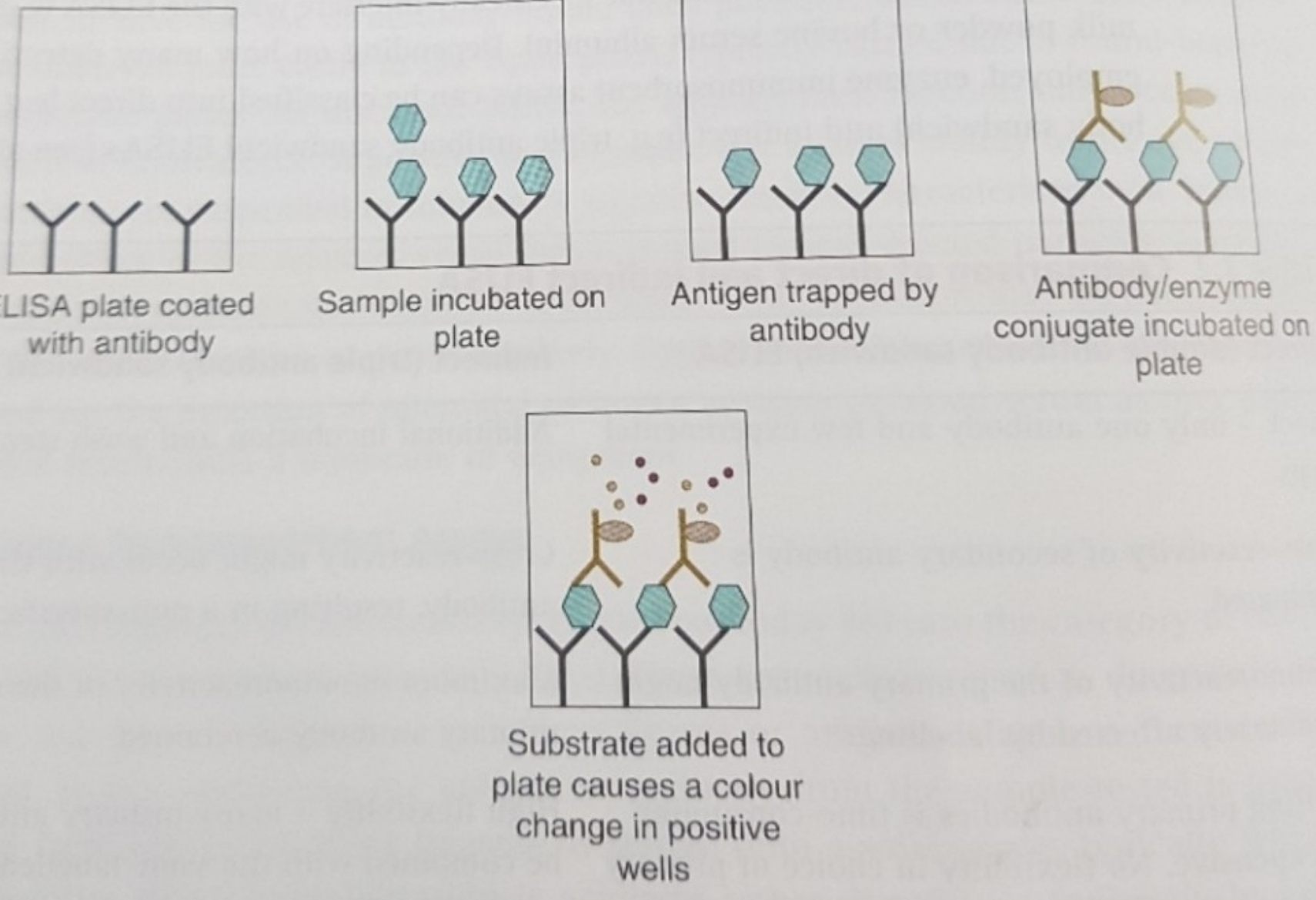


Figure 7.12 Schematic diagram of the double antibody sandwich (DAS) ELISA.

Triple Antibody Sandwich ELISA

- Also known as **indirect ELISA**, is a method often used to **identify antibodies against pathogens in patient blood** to diagnose infection.
- As an example of a diagnostic test, **HBV capture antibody** is bound to the well of microtitre plate and **coat protein** from the virus (the antigen) is added.
- After incubation and washing, **patient serum** is added, which ,if it contains antibodies, reacts with antigen.
- **Anti-human antibody conjugate to an enzyme** marker is added to identify the samples which are positive for HBV antigen
- The test works well for the **diagnosis of HBV infection** and is also used to ensure that **blood donations given for transfusion are free from this virus.**

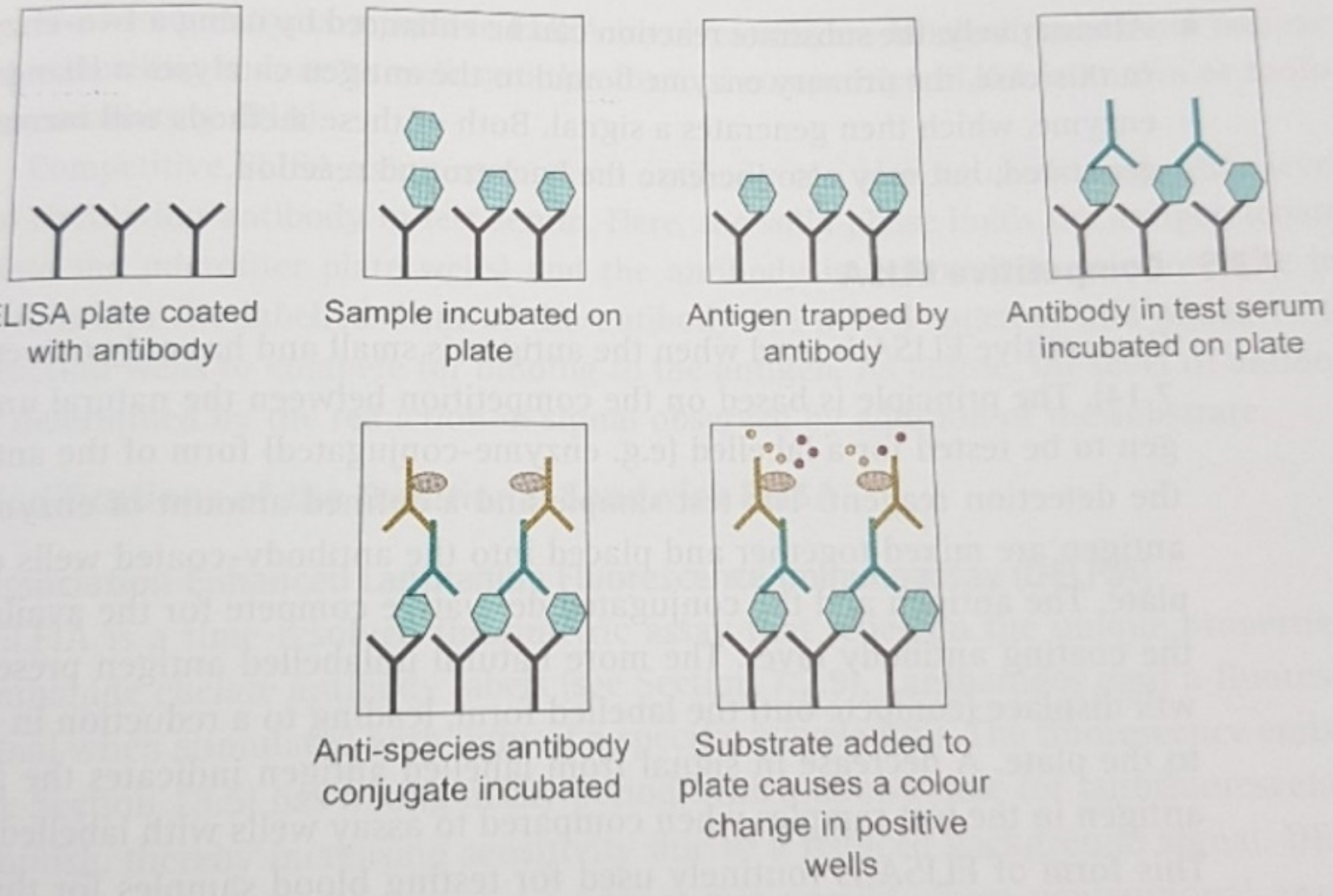


Figure 7.13 Schematic diagram of a triple antibody sandwich (TAS) ELISA.

Competitive ELISA

- The principle is based on the **competition between the natural unlabelled antigen to be tested AND a labelled (enzyme-conjugate) form of the antigen** .
- The test sample and a defined amount of enzyme-conjugated antigen are mixed together and placed in to antibody-coated wells of a microtitre plate
- The antigen and the conjugated derivative compete for available spaces on the coated antibody layer
- The more natural unlabelled antigen present, the more it will displace the labelled form, leading to a reduction in enzyme bound to plate
- **Increased serum antigen results in reduced binding of the antigen-enzyme conjugate with the capture antibody producing less enzyme activity and reduced color formation.**

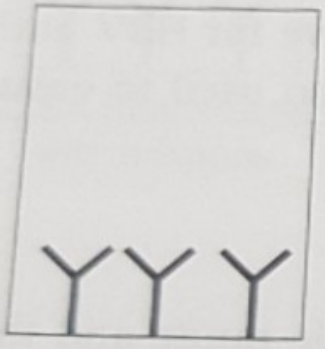
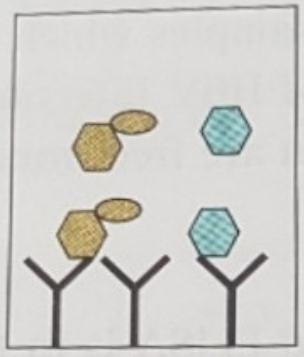
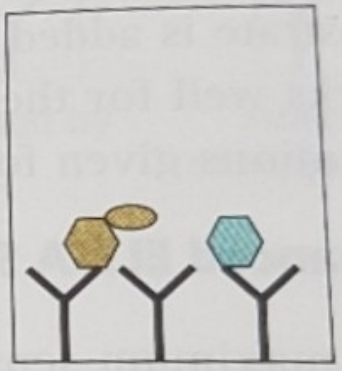


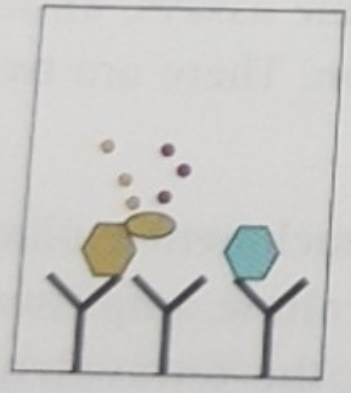
Plate coated with antibody



Sample antigen and conjugated antigen added



Native and conjugated antigen compete for coating antibody



Substrate added to plate causes a colour change in positive wells

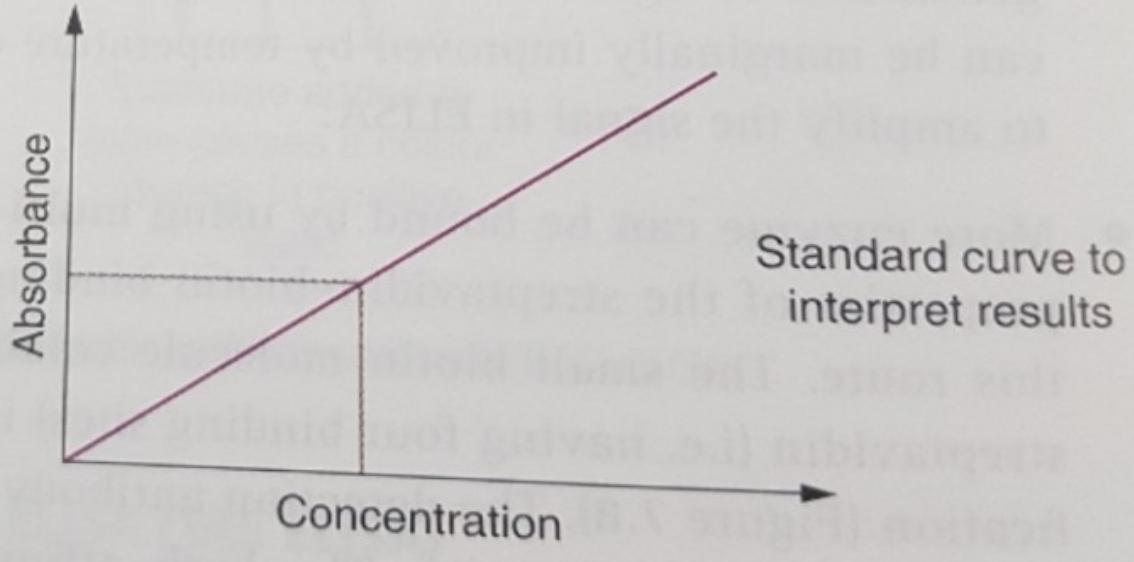
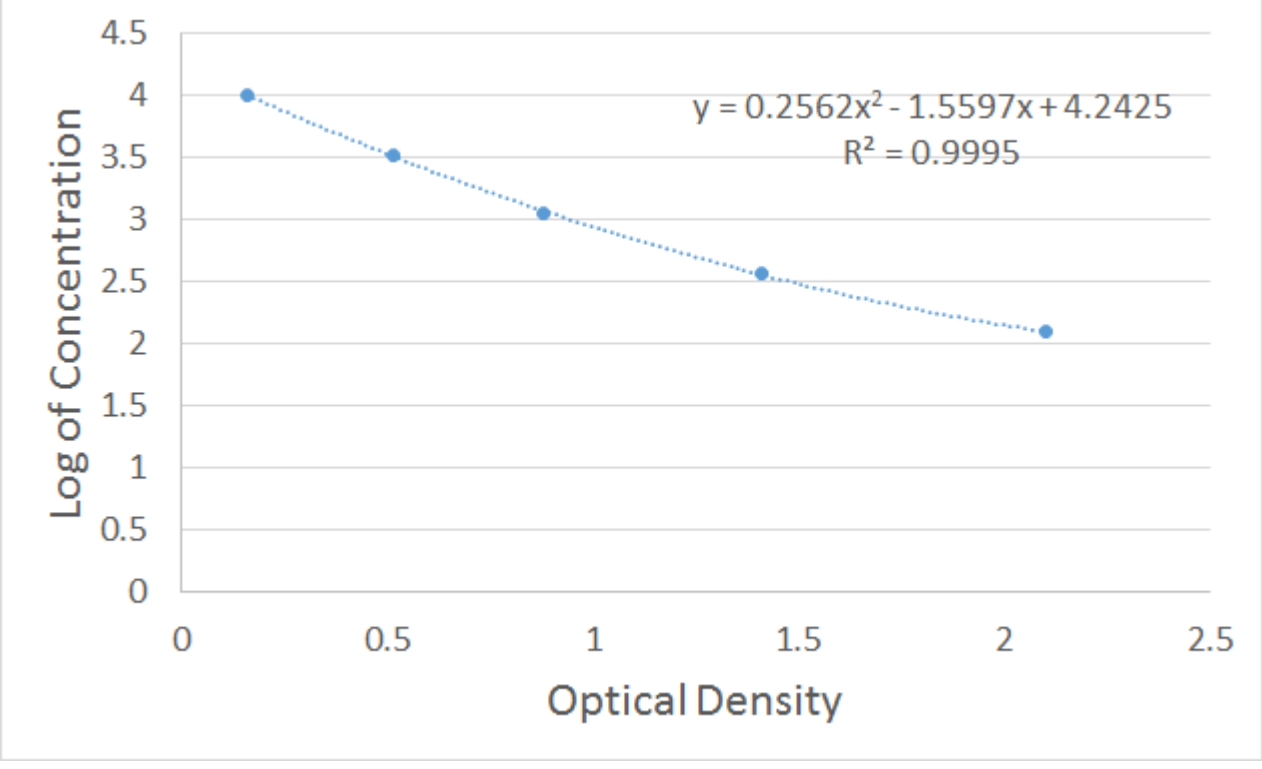


Figure 7.14 Competitive ELISA.



- This form of ELISA is routinely use for **testing blood samples for thyroxine**

- Competitive ELISA provide an **accurate measure of the circulating level of the hormone** compared to standard curve of known dilutions

APPLICATIONS

- Screening donated blood for evidence of **virus contamination** by

  HIV-1 and HIV-2(presence of anti-HIV antibodies)

  Hepatitis C (presence of antibodies)

  Hepatitis B (presence of both antigen and antibodies)

- Measuring hormone levels of **HCG,LH ,TSH,T_3 and T_4**

- Detecting infections like **HIV, syphilis and chlamydia or Hepatitis B and C.**