



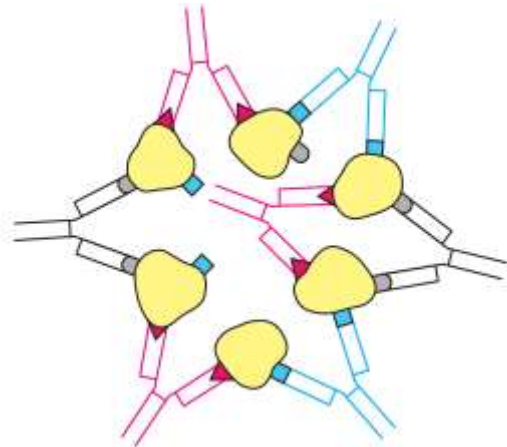
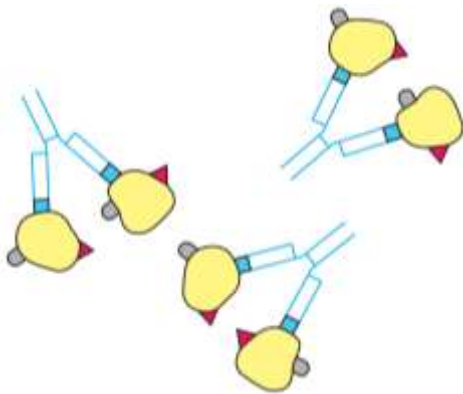
RIA & ELISA

Objectives

- Principle of immunoassay
- Types of immunoassays
- Principle of radioimmunoassay & ELISA
- Various types of ELISA
- Steps of ELISA
- Urine pregnancy test by lateral flow immunoassay

Immunoassay

- Immunoassay is an analytical technique used for the quantification of an analyte based on the antigen-antibody reaction.



Labels in immunoassay

- The advantage of immunoassay is that either the antigen or antibody can be labelled.
- This label can be an enzyme, radioactive compound, chemiluminescent or fluorescent.
- The labelled component of an immunoassay is sometimes called the tracer.

Types of immunoassay

- Radioimmunoassay (RIA)
- Enzyme-linked immunosorbent assays (ELISA)
- Fluoroimmunoassay (FIA)
- Chemiluminescence immunoassay (CLIA)

Constituents of labelled immunoassay

- Specific antibody/antibodies (labelled)
- Antigen (known/unknown)
- A method to separate the unbound components from bound immune complex
- A method for detection of the label
- Standards

Radioimmunoassay



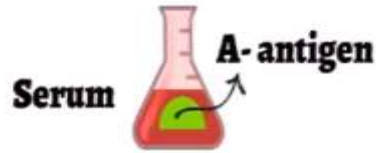
**Dr. Rosalyn Yalow and Dr. Solomon
Berson**



**Physiology or Medicine
1977**

RIA-Principle

- Competitive binding assay
- “Radio labelled antigen (Ag^*) competes with the unlabelled antigens for the binding site of the fixed amount of antibody (Ab).”



**Anti A
Antibodies**



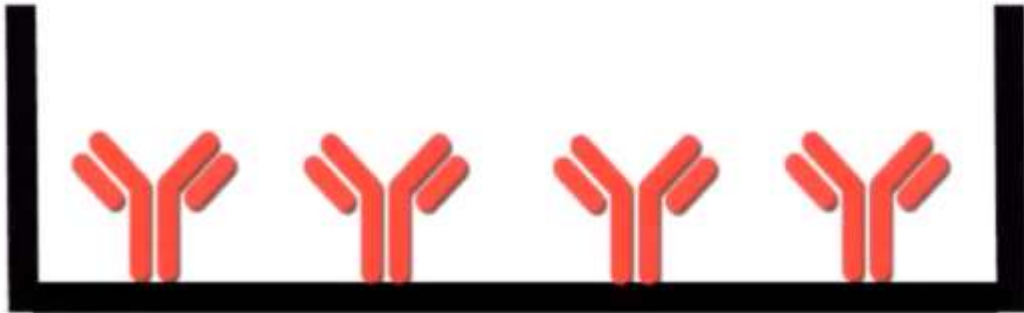
**Radiolabeled
A antigen**



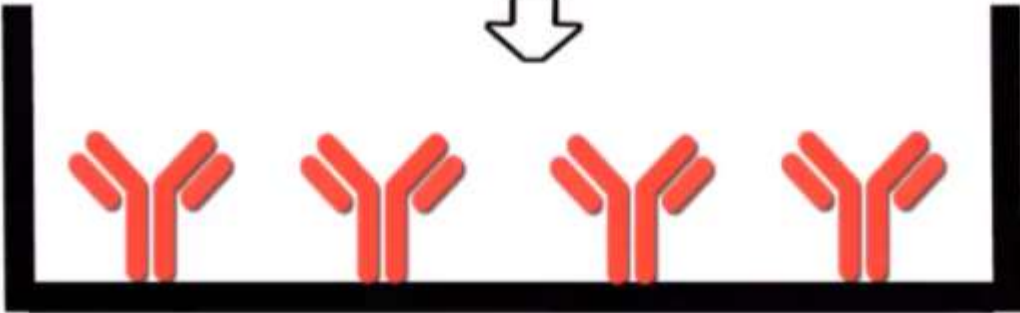
**Unlabeled
A antigen**



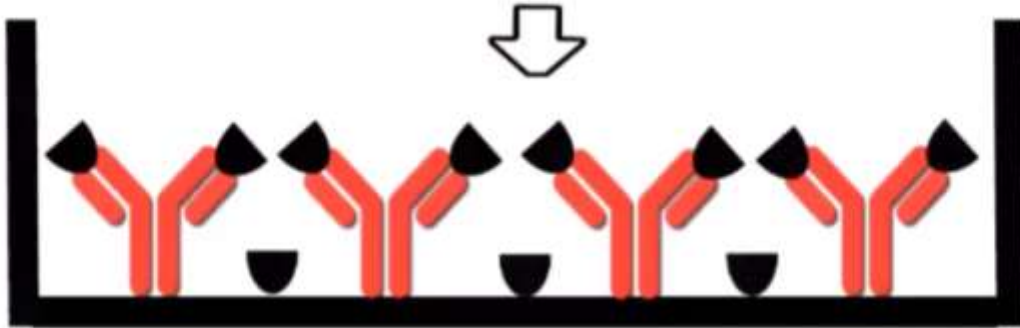




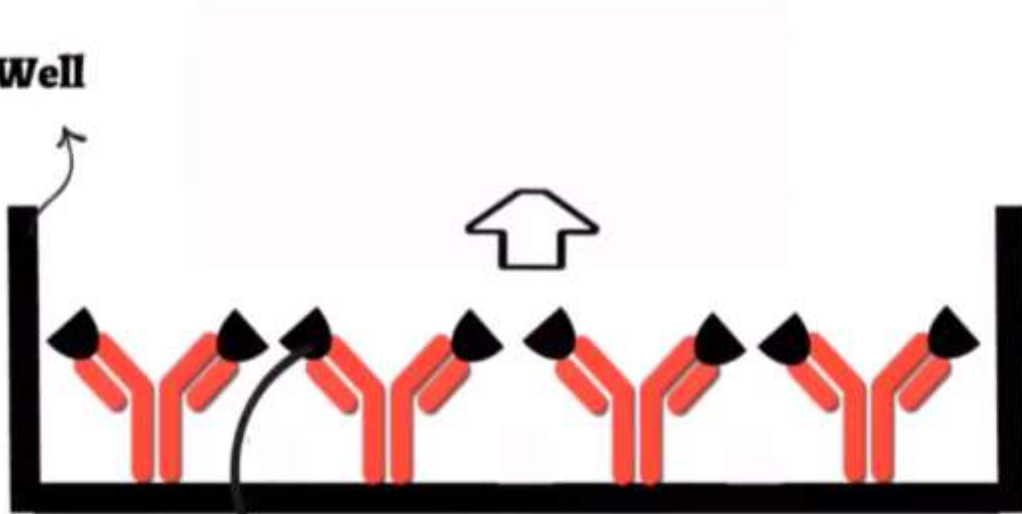
**Radiolabeled Ag
in excess**



**Radiolabeled Ag
in excess**



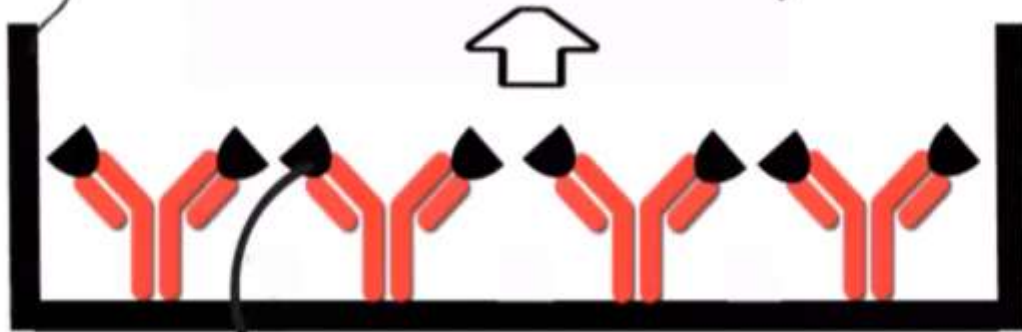
Well



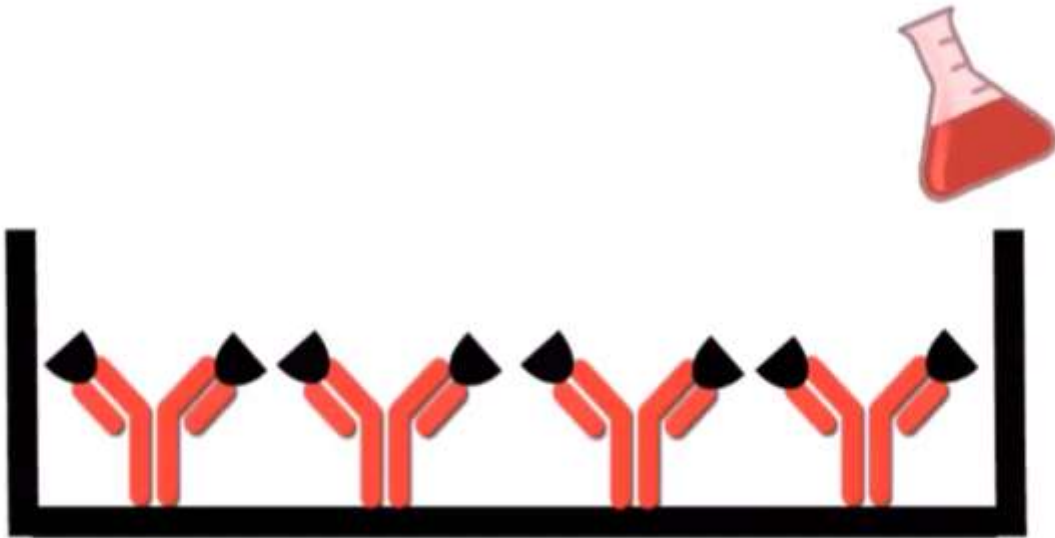
**Radiolabeled
antigen**

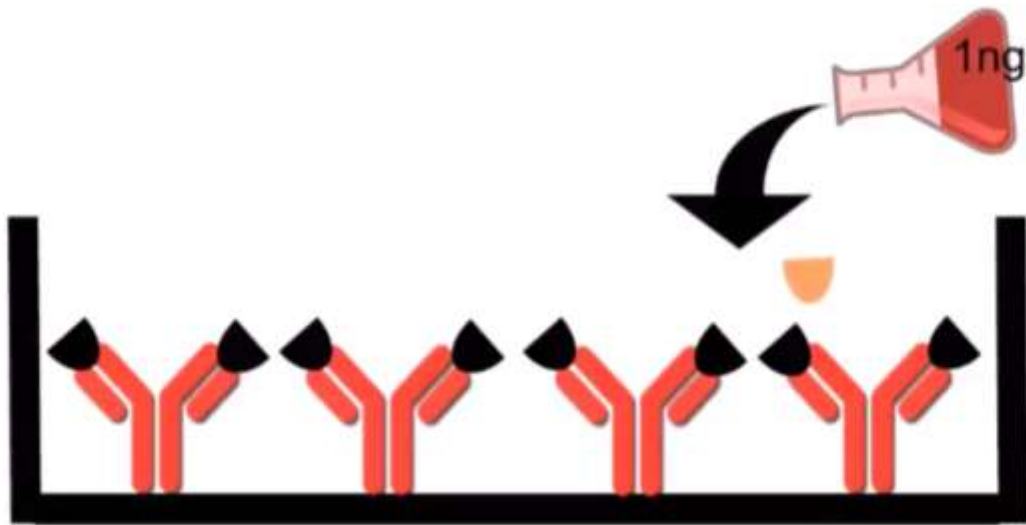
Well

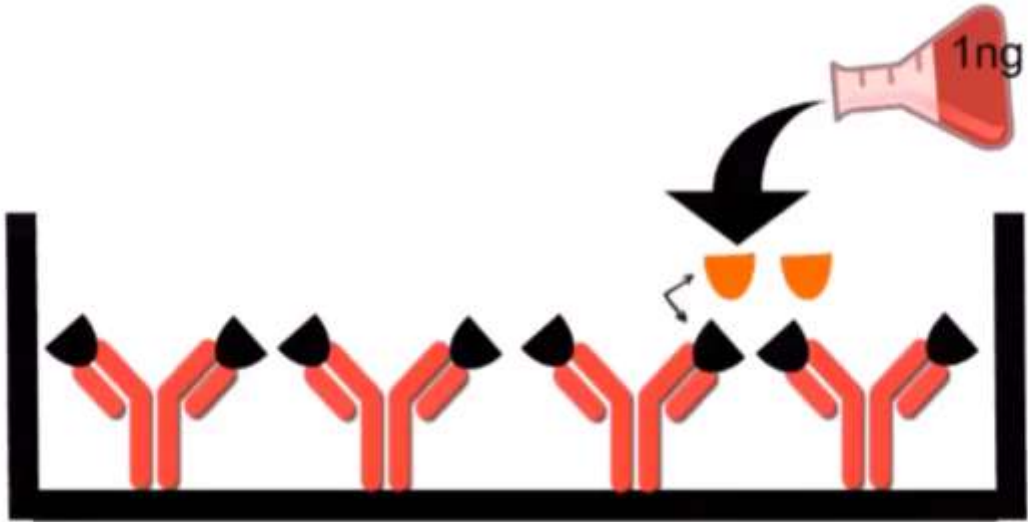
100% Radioactivity



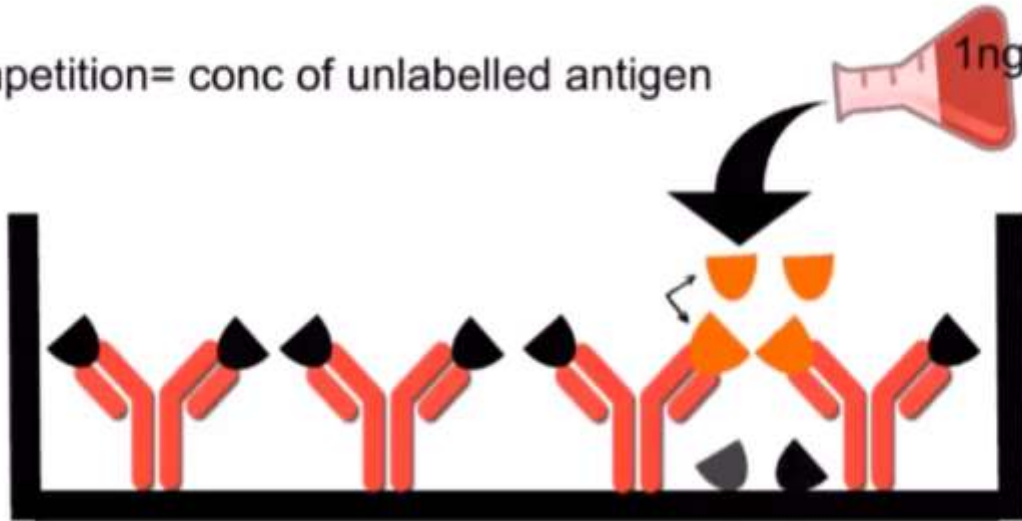
**Radiolabeled
antigen**



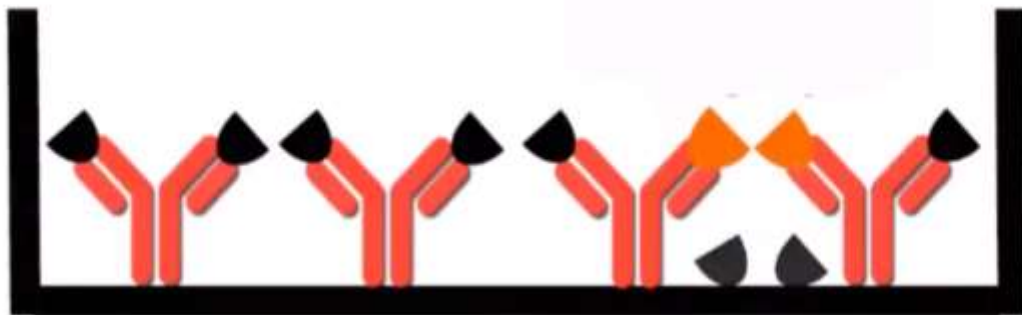




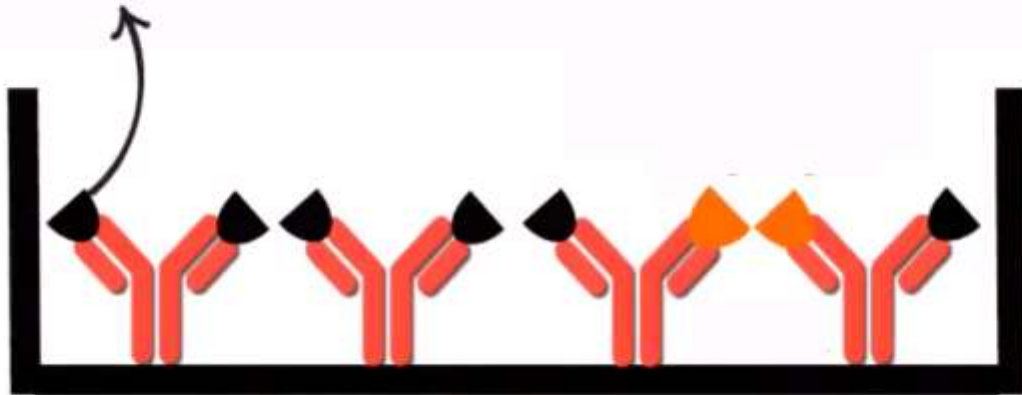
competition= conc of unlabelled antigen



Washing

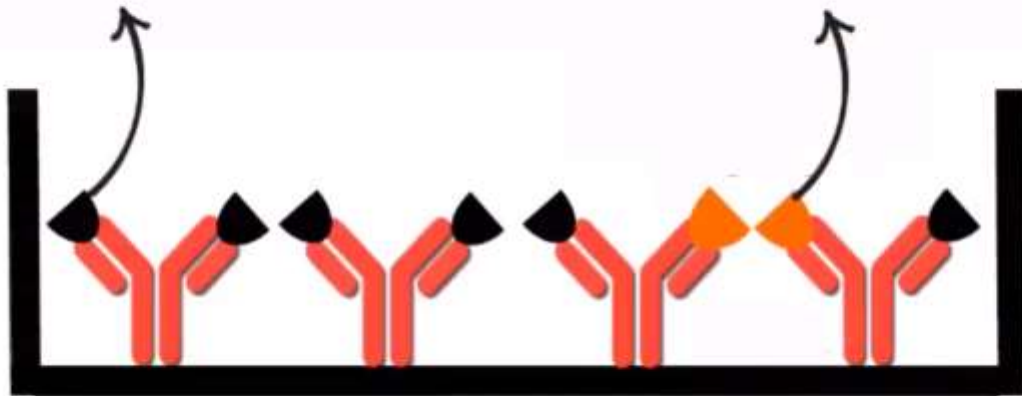


Radiolabeled Ags decreased



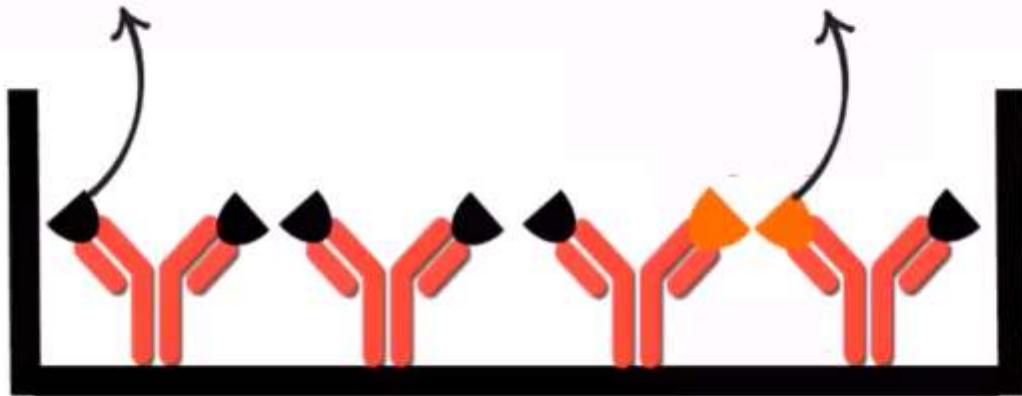
Radiolabeled Ags decreased

Unlabeled Ag



Radiolabeled Ags decreased

Unlabeled Ag

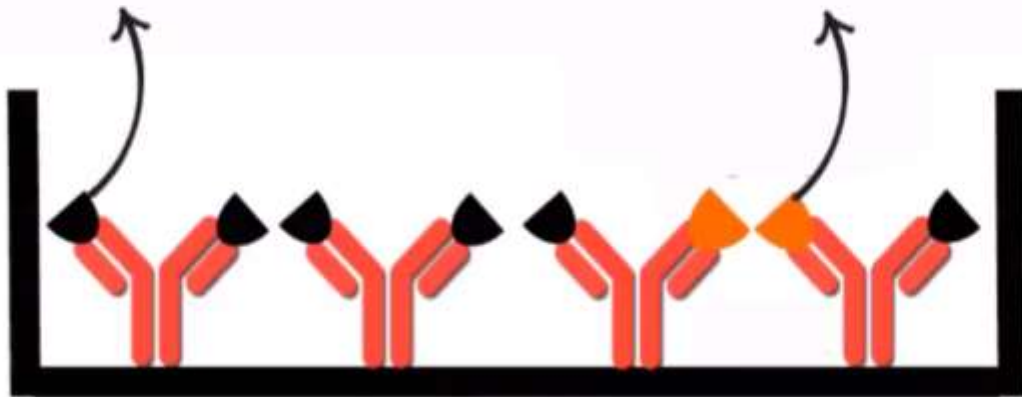


0 ng - 100%

1 ng

Radiolabeled Ags decreased

Unlabeled Ag



0 ng - 100%

1 ng - 90%

Unlabeled A	% Radioactivity
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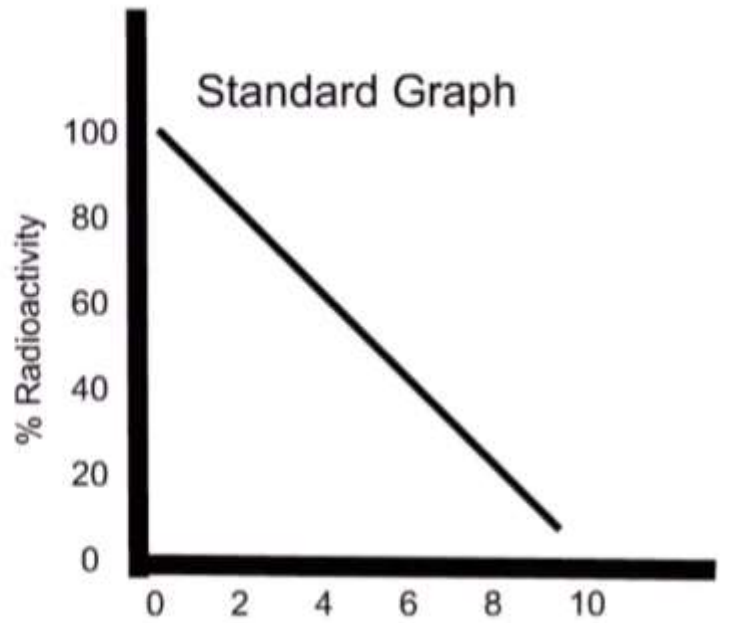
0	100
---	-----

1	90
---	----

-	-
---	---

0	100
---	-----

0	100
---	-----



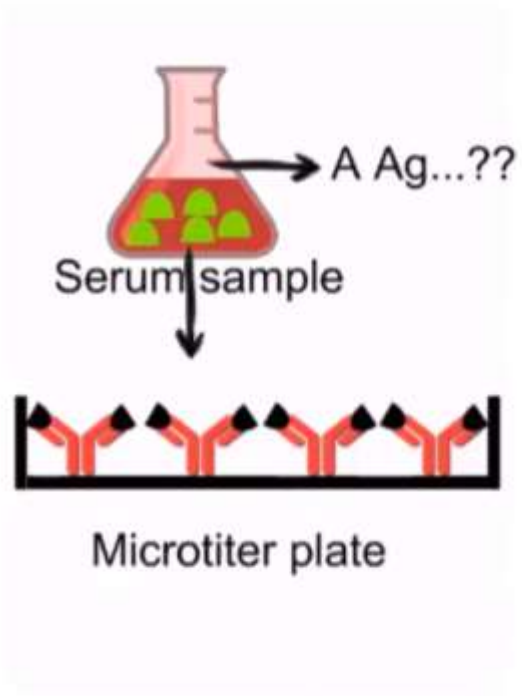


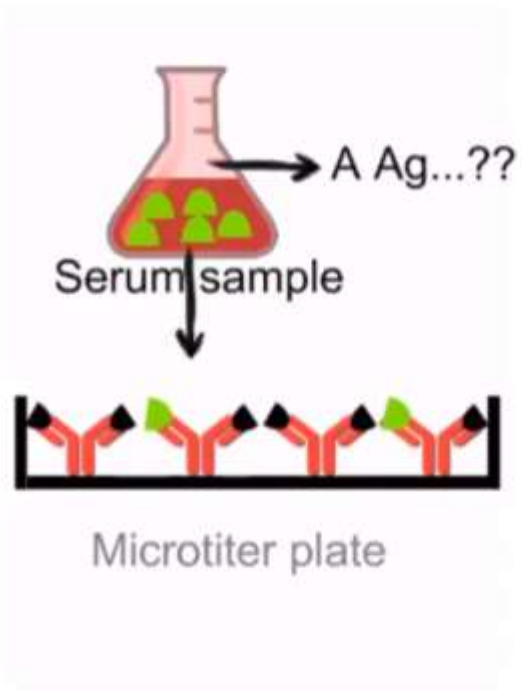
Serum sample

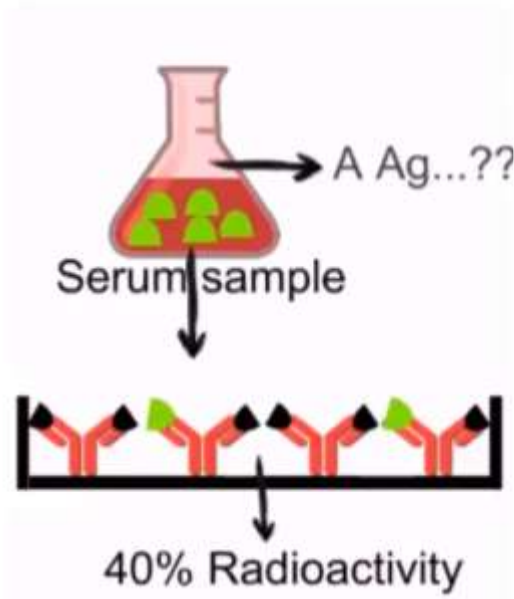
→ A Ag...??



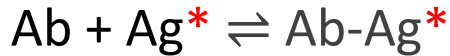
Microtiter plate



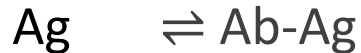




RIA- Principle:



+



The proportion of Ab-Ag^* and Ab-Ag at equilibrium will be equal to the original proportion of Ag^* to Ag

Disadvantages of RIA

- Very high cost of equipments and reagents.
- Half life of I^{125} is 60 days only.
- Hazards of radioactivity.
- Lengthy procedure.

Enzyme-Linked Immunosorbent Assay

- ELISA is the simplified and modified version of RIA.
- Instead of the radiolabelled antibodies, enzyme-linked antibodies are used in ELISA.
- It eliminates all the hazards associated with radioactivity and the equipment needed for measuring radioactivity.

Types of ELISA

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

Direct ELISA



Matrix or Surface

Direct ELISA

Advantages:

- Quick because only one antibody and fewer steps are used.

Direct ELISA

Disadvantages:

- Immunoreactivity of the antibody might be affected by labeling.
- Labelling primary antibodies for each specific ELISA system is time-consuming and expensive.
- Less signal amplification.

Indirect ELISA



Antigen-coated well



Indirect ELISA - Advantages

- Versatile - any primary antibodies can be made in one species and the same labelled secondary antibody can be used for detection.
- Maximum immunoreactivity of the primary antibody is retained.
- Sensitivity is increased because of signal amplification.

Signal-Amplification in ELISA

- Enzyme labelled secondary antibodies are responsible for the signal amplification.
- Each primary antibody can be bound by more than one secondary antibody.
- Similarly, each enzyme molecule will process multiple substrates into the product.
- This leads amplification at each step, responsible for high sensitivity of the ELISA.

Sandwich ELISA



Sandwich ELISA



Monoclonal antibody-coated well



Sandwich ELISA

- The antigen is sandwiched between two primary antibodies recognizing two different epitopes of the antigen.
- The first antibody is coated on the plates.
- The second antibody, which is added after the antigen addition, is linked to the enzyme. It is an Ag detection test.
- The first antibody is known as 'capture' antibody and the second antibody is known as 'detection' antibody.

Sandwich ELISA

- It is important to note that the antibodies used should be able to recognise the different epitopes of the same antigen.
- So, haptens can't be detected using sandwich ELISA.


Competitive ELISA

- Competitive ELISA is similar to RIA.
- Both unknown antigen (sample) and the known antigen (standard) compete with each other for a fixed amount of antibody.
- Ag is detected.
- Normally used for hapten detection.

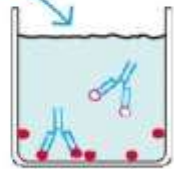
Competitive ELISA

Competitive ELISA

Incubate antibody with antigen to be measured

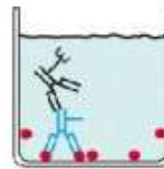


Add Ag-Ab mixture to antigen-coated well



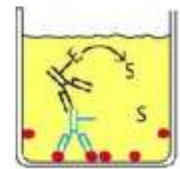
wash

Add enzyme-conjugated secondary antibody



wash

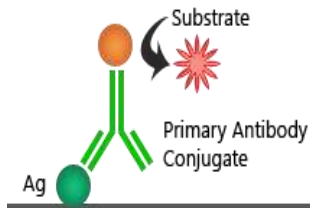
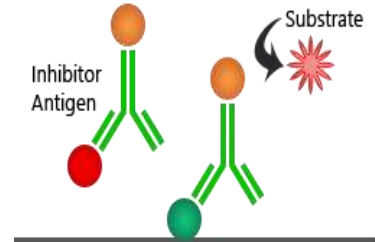
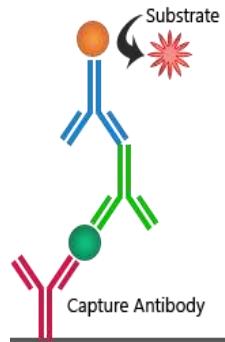
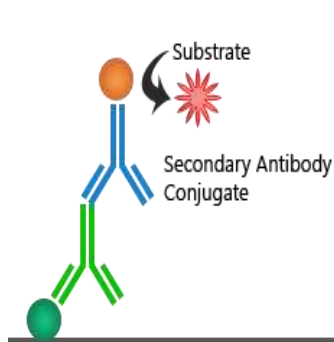
Add substrate and measure color



Comparison of the 4 types of ELISA

Type of ELISA	Well coated with	Detects in serum	Example
Direct	Antigen	Antigen	Beta hCG
Indirect	Antigen	Antibody	Anti HBV antibody
Sandwich	Antibody	Antigen	HIV: p24 antigen
Competitive	Antigen	Antigen	Hapten detection

Identify the type of ELISA:



Which ELISA will you use?

- Histamine - Competitive
- Complement C3 - Competitive
- Insulin - Sandwich
- Interleukins - Sandwich
- Leukotrienes - Competitive
- Hepatitis B surface antigen - Sandwich
- Dengue IgM Antibodies - Indirect

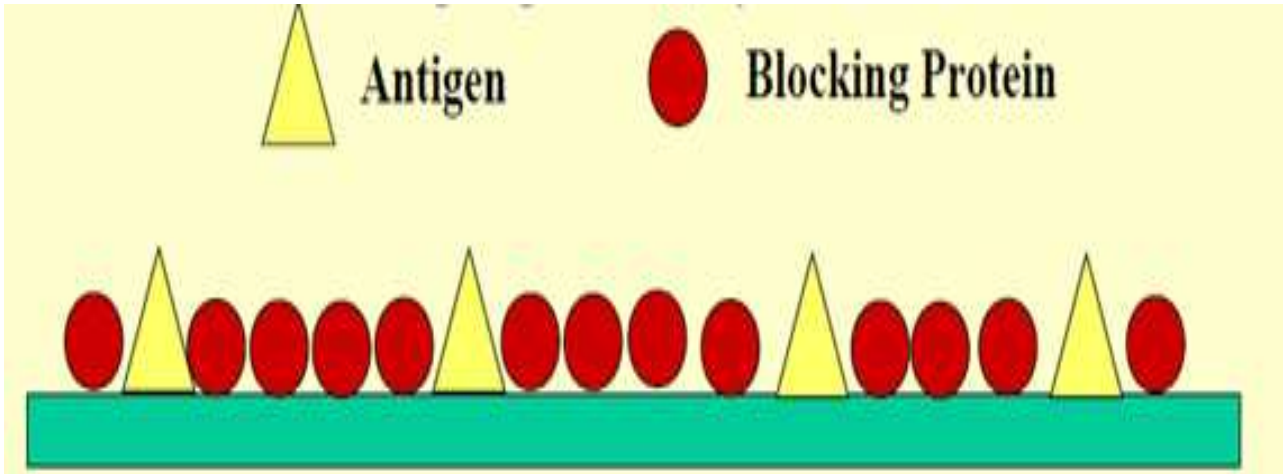
Steps of ELISA

1. Coating
2. Blocking
3. Addition of Antibodies
4. Washing
5. Detection

Coating



Blocking



Blocking agents

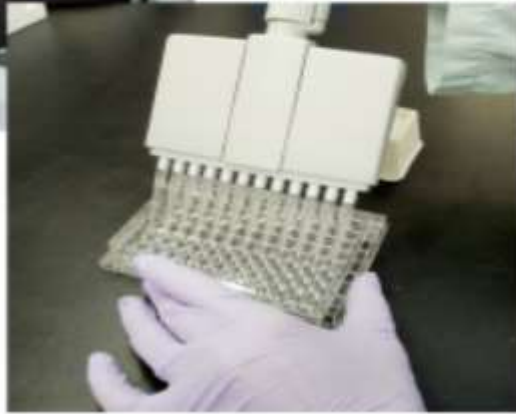
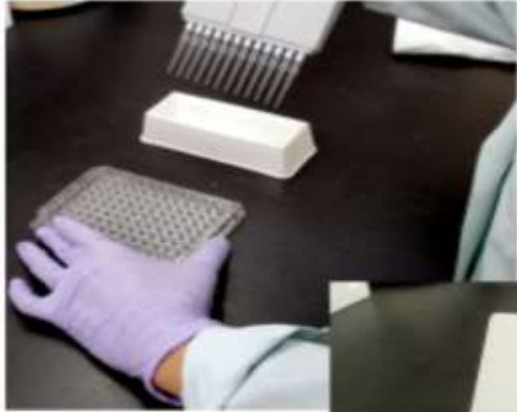
- 1% Bovine serum albumin (BSA)
- Serum
- Non-fat dry milk (NFM)
- Casein or Gelatin in PBS

96-well plate ▼

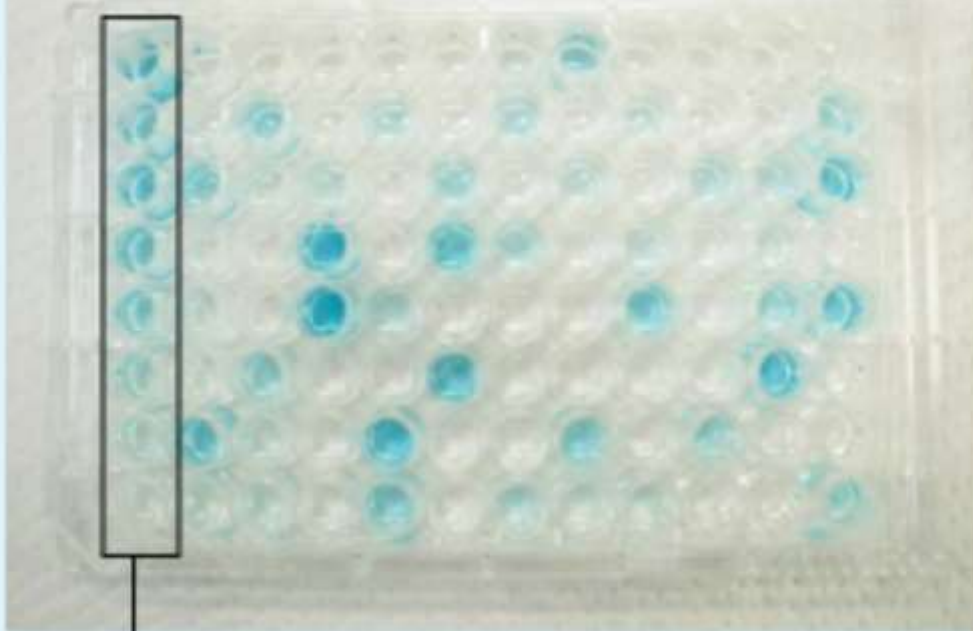
▼ Reservoir



▲ 12-Channel pipette

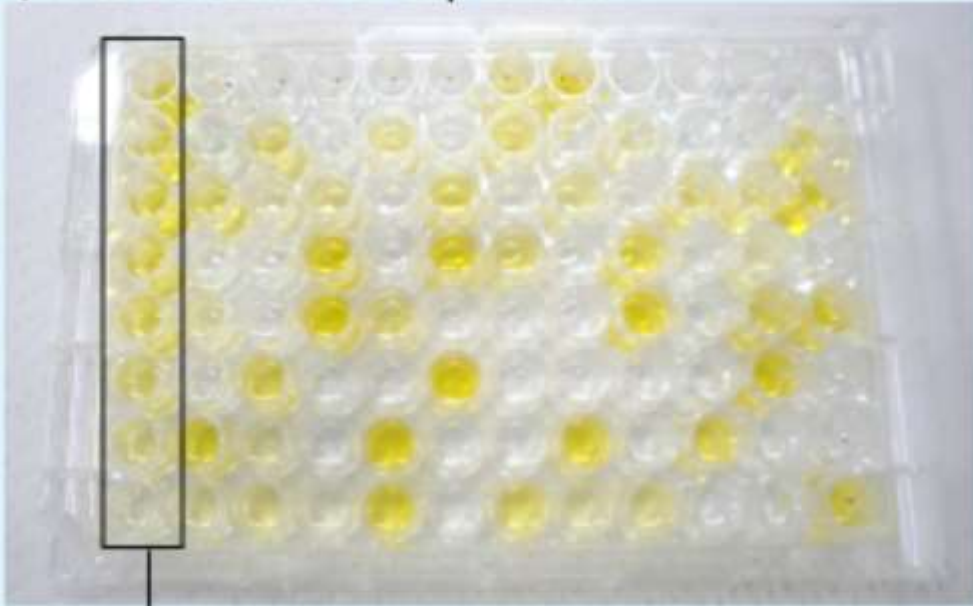


10 minutes after the addition of the substrate



Dilution series of a standard for the calibration curve

▼ After the addition of stop solution



Dilution series of a standard for the calibration curve

Plate reader ▼



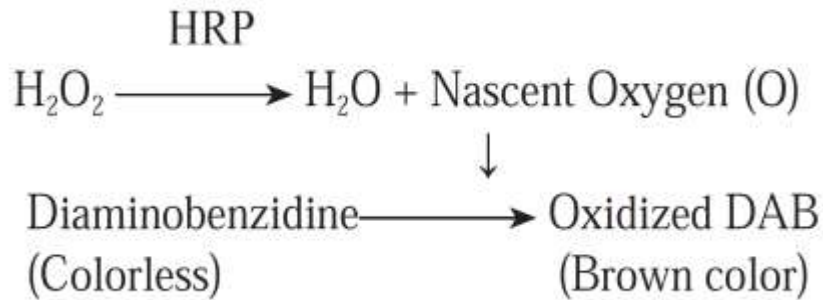
Detection

- The final stage in all ELISA systems is a detection step.
- This involves the introduction of an enzyme-substrate system.
- The enzyme converts the substrate to a detectable product.

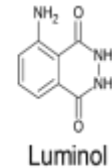
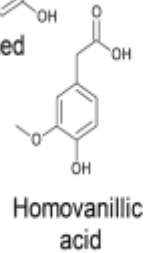
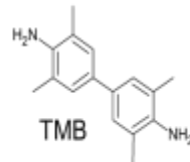
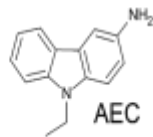
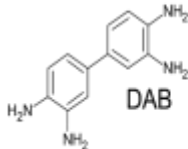
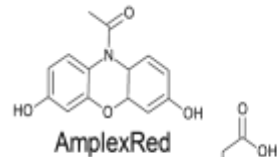
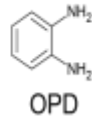
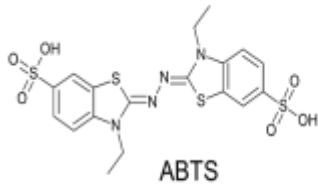
Commonly used enzymes

- Horseradish peroxidase
- Alkaline phosphatase

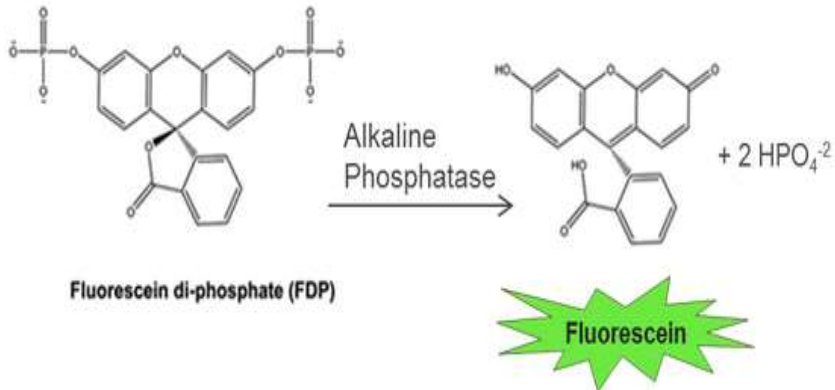
Horse-radish peroxidase



Chromogenic Substrate



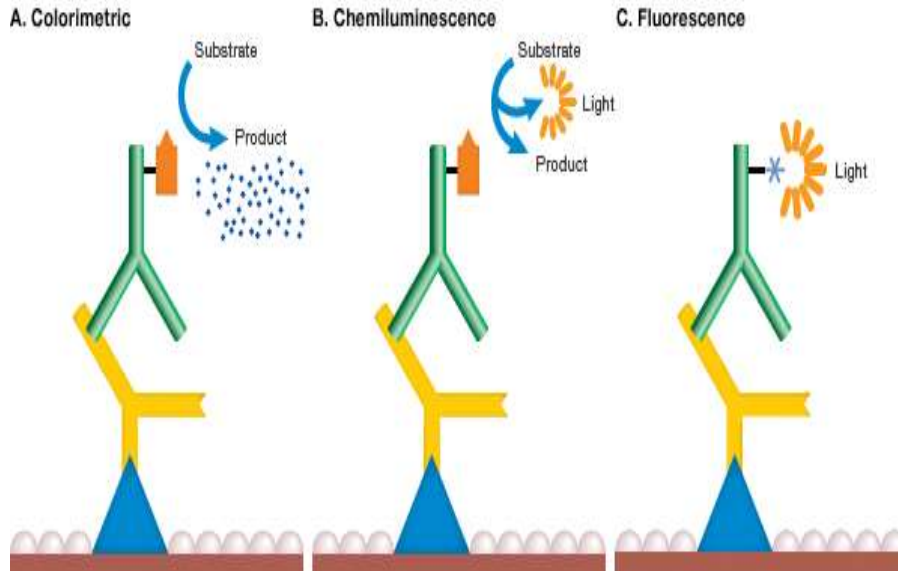
Alkaline phosphatase



ALP chromogenic substrate

1. Nitrophenyl phosphate (NPP)
2. p-nitrophenyl pyrophosphate (PNPP)
3. Bromo chloro indolyl phosphate (BCIP) & Nitro blue tetrazolium (NBT)
4. Fluorescein diphosphate

Various types of detection



URINE PREGNANCY TEST

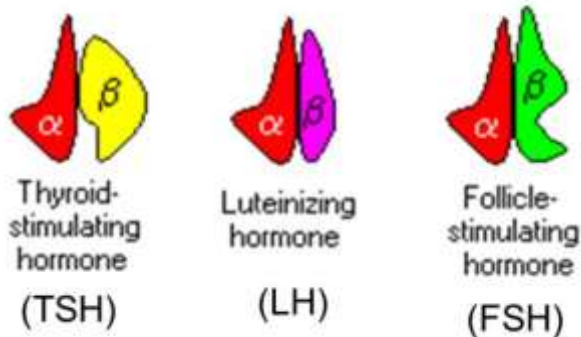
Point-of-care-testing



Human chorionic gonadotrophin

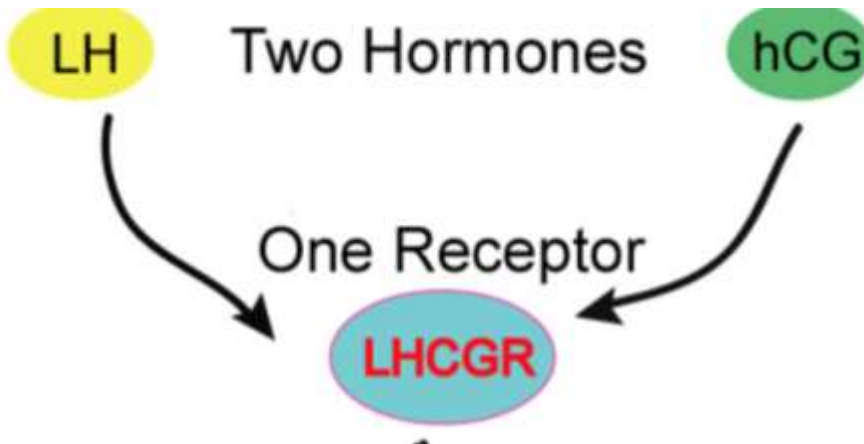
- hCG is synthesized in the syncytiotrophoblast cells of the placenta.
- Minute amounts are also made in the pituitary.
- A single gene located on chromosome 6 encodes the α subunit.
- Chromosome 19 contains genes for beta subunit.

Alpha subunit is shared



Alpha subunit is shared by glycoprotein hormones (TSH, LH [luteinizing hormone], FSH, and hCG).

hCG receptor

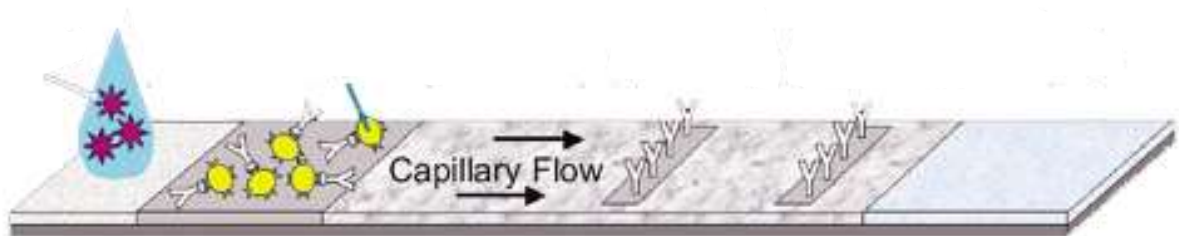


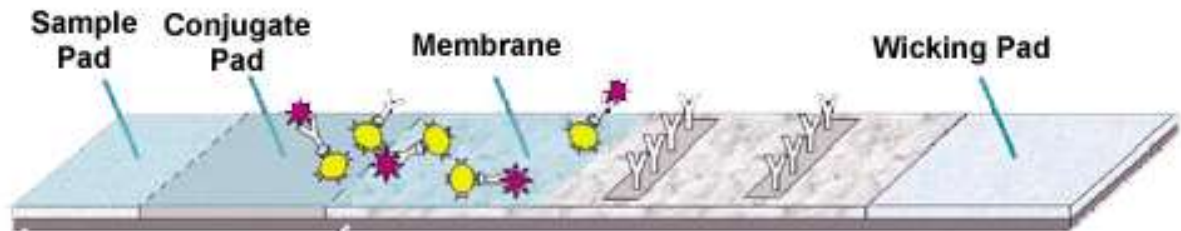
hCG and pregnancy

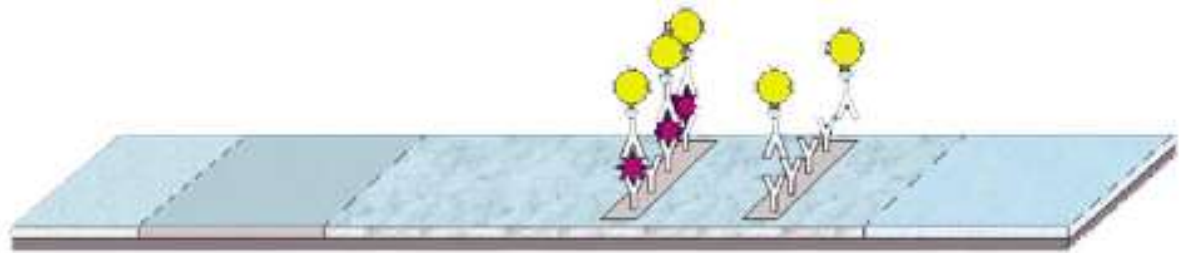
- Synthesis of hCG β peaks at about 8 to 10 weeks of gestation.
- First-morning specimens are preferred for qualitative urine pregnancy tests because they are concentrated and contain abundant hCG.

Lateral flow Immunoassay

Lateral flow Immunoassay







Test Line (Positive) **Control Line (Valid Test)**



Interpret:



Thank You!

