



# RIA & ELISA

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# Objectives

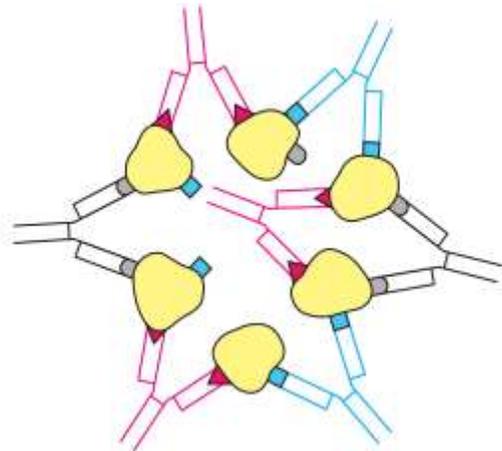
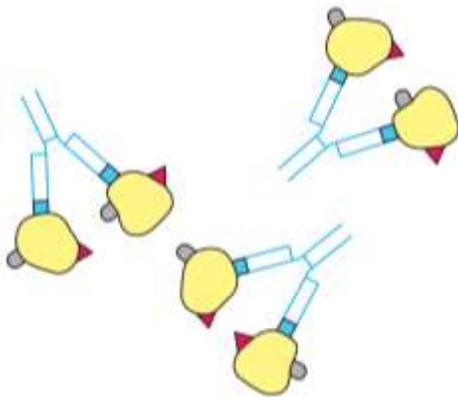
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- Principle of immunoassay
- Types of immunoassays
- Principle of radioimmunoassay & ELISA
- Various types of ELISA
- Steps of ELISA
- Urine pregnancy test by lateral flow immunoassay

# Immunoassay

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



# Labels in immunoassay

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- 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

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

# Constituents of labelled immunoassay

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- 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

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

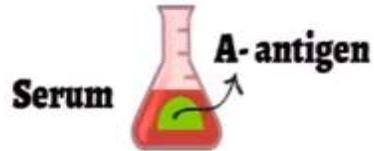


**Physiology or Medicine  
1977**

# RIA-Principle

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



**Anti A  
Antibodies**



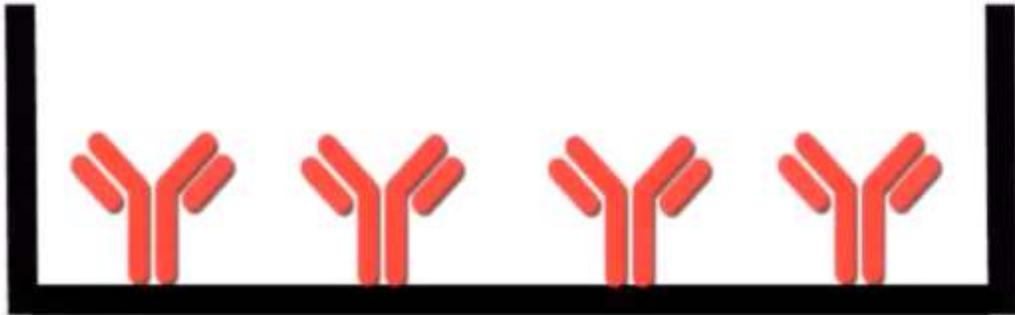
**Radiolabeled  
A antigen**



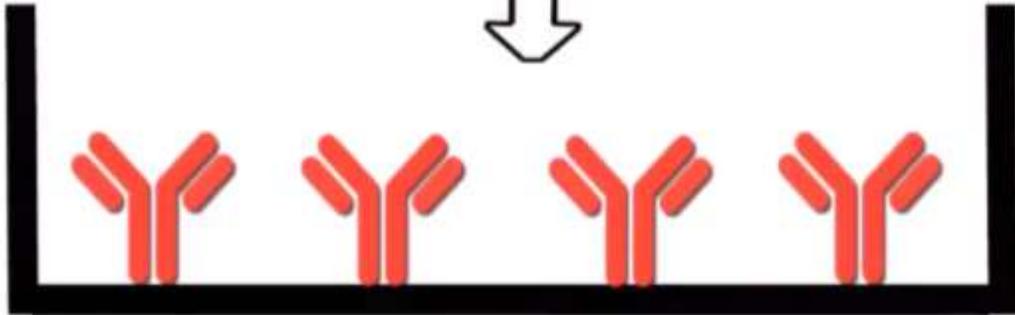
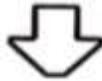
**Unlabeled  
A antigen**



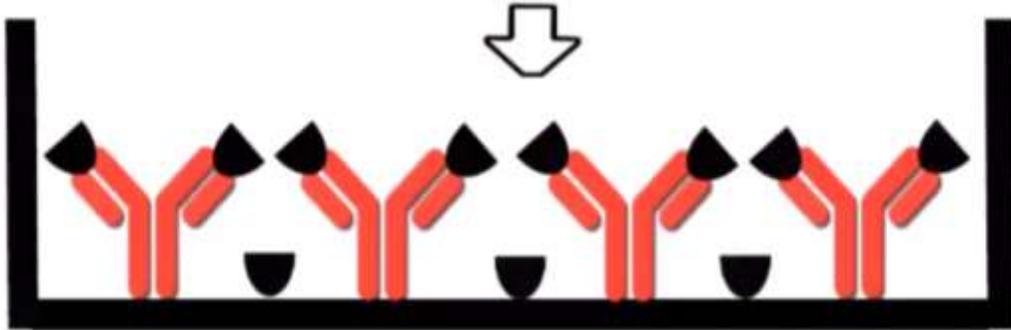




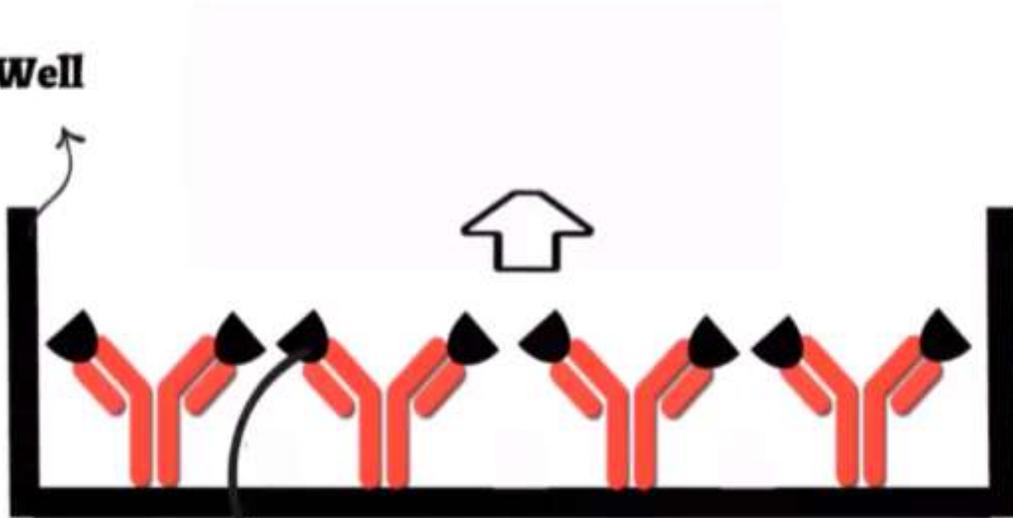
**Radiolabeled Ag  
in excess**



**Radiolabeled Ag  
in excess**



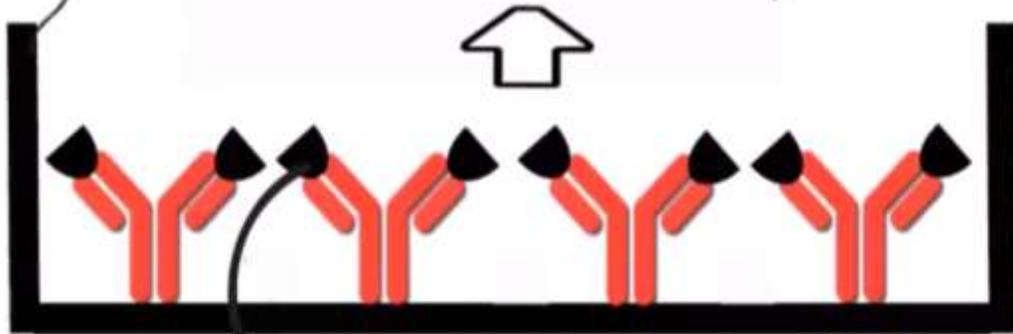
**Well**



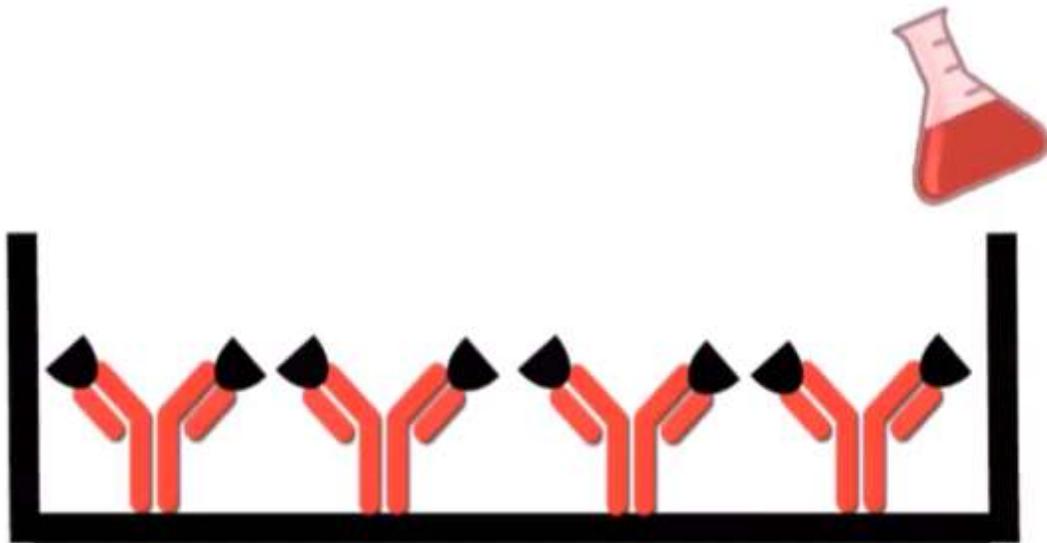
**Radiolabeled  
antigen**

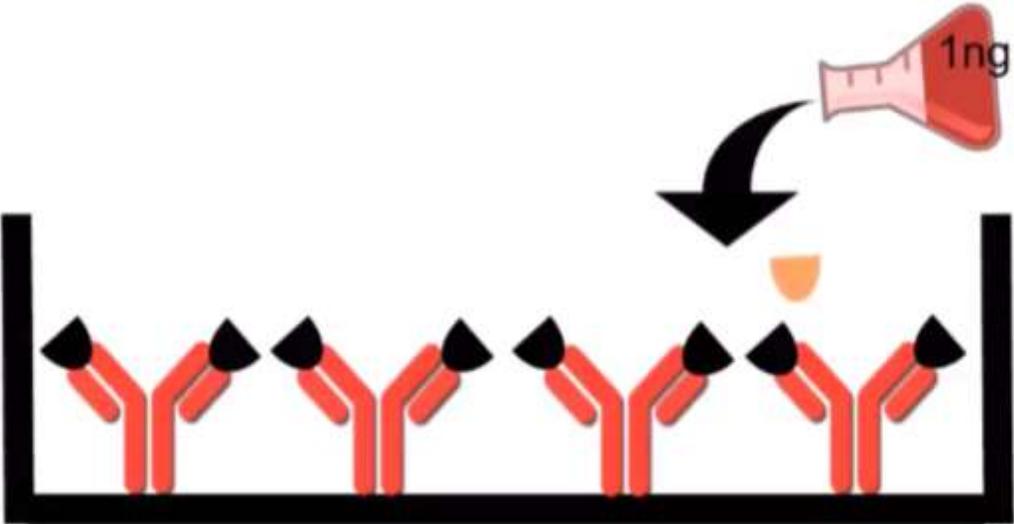
Well

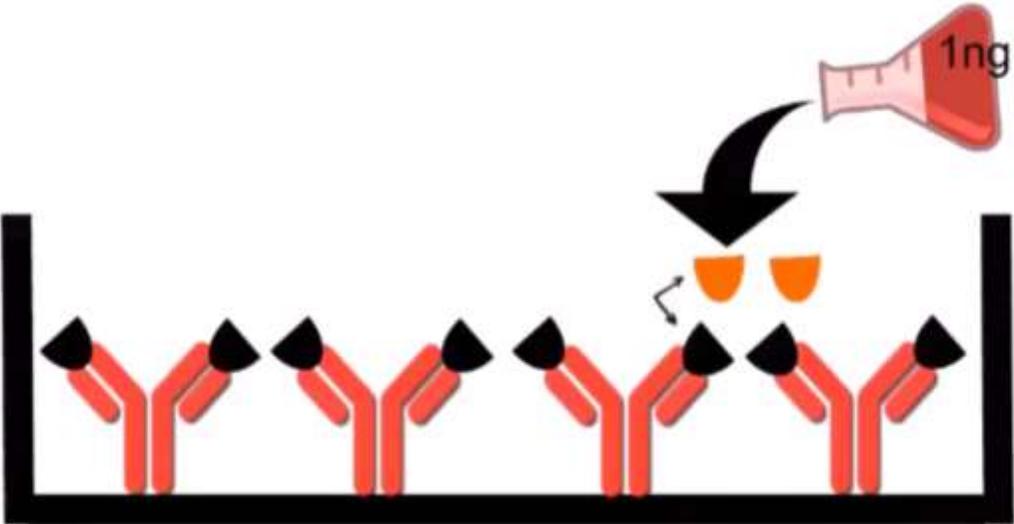
**100% Radioactivity**



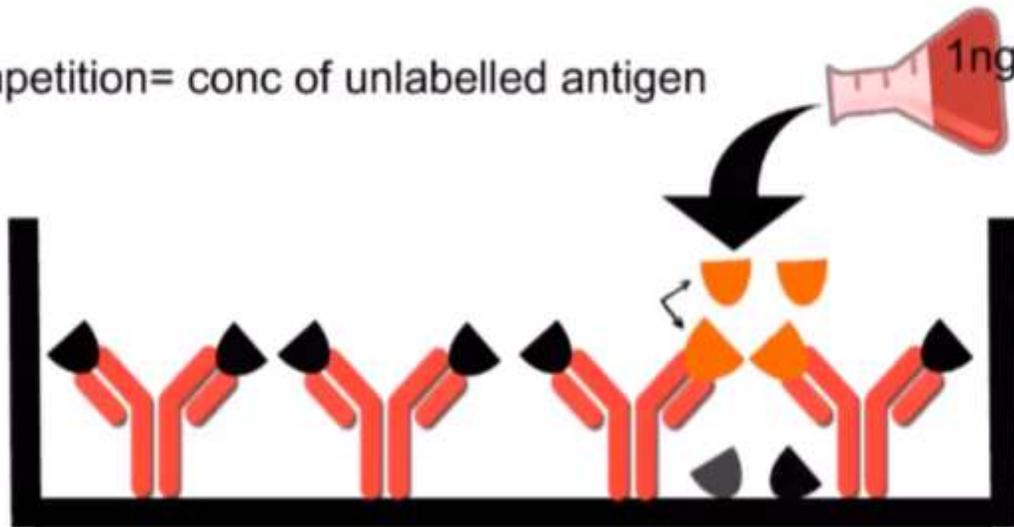
**Radiolabeled  
antigen**



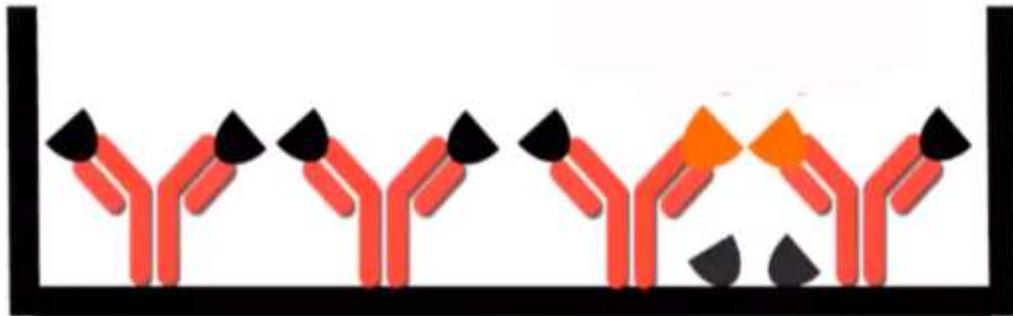




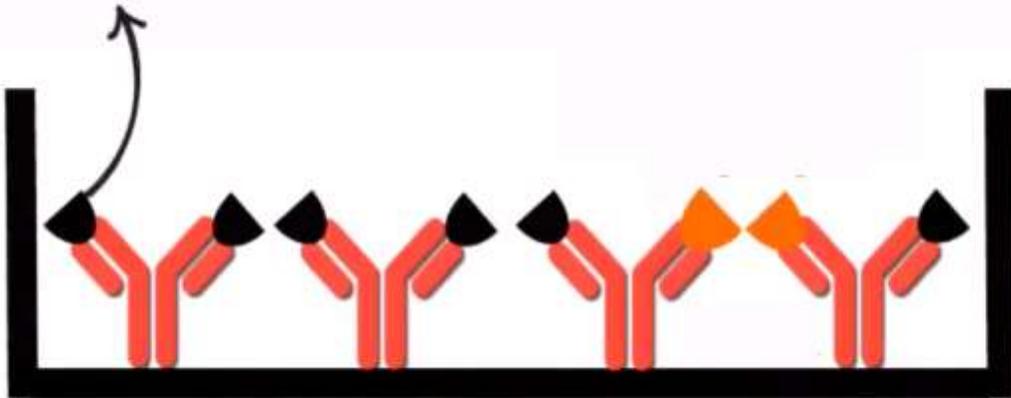
competition= conc of unlabelled antigen



# Washing

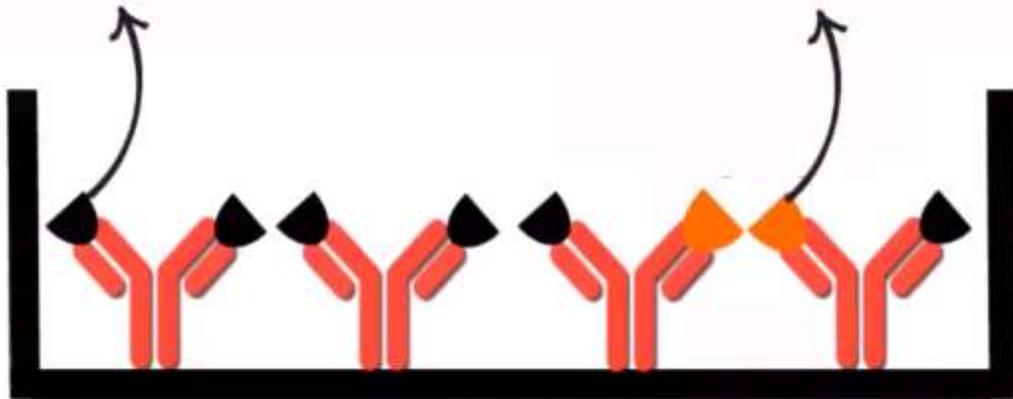


Radiolabeled Ags decreased



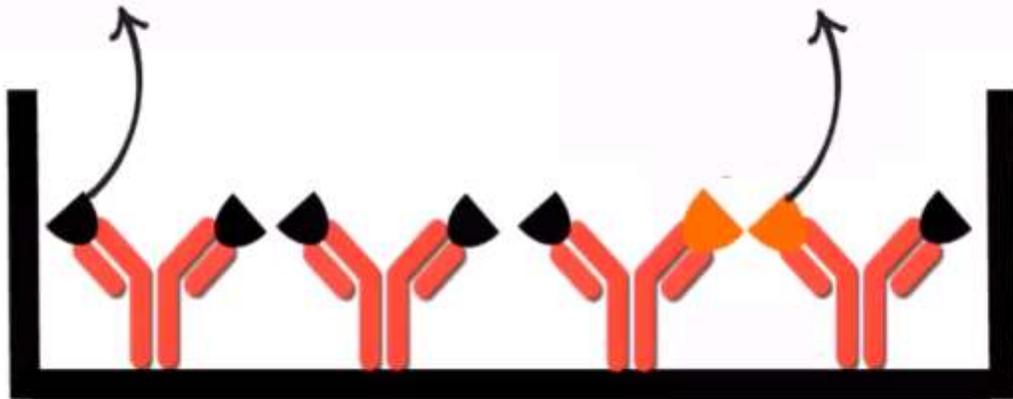
Radiolabeled Ags decreased

Unlabeled Ag



Radiolabeled Ags decreased

Unlabeled Ag

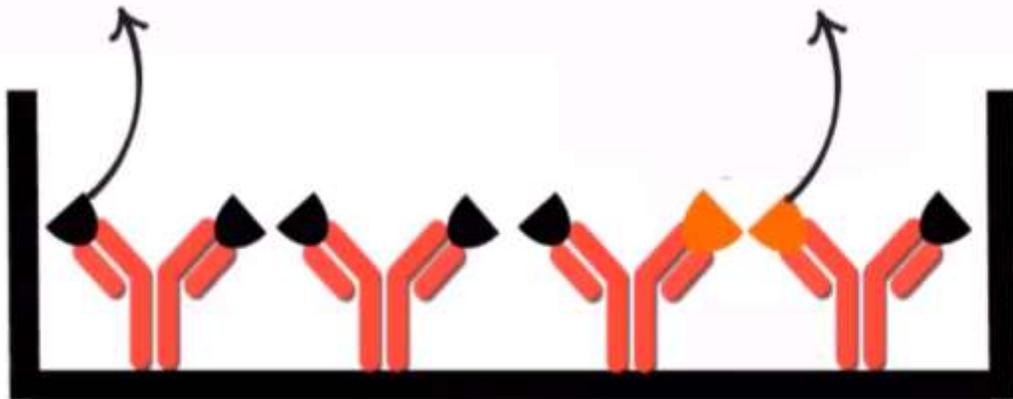


0 ng - 100%

1 ng

Radiolabeled Ags decreased

Unlabeled Ag



0 ng - 100%

1 ng - 90%

| Unlabeled<br>A | %<br>Radioactivity |
|----------------|--------------------|
|----------------|--------------------|

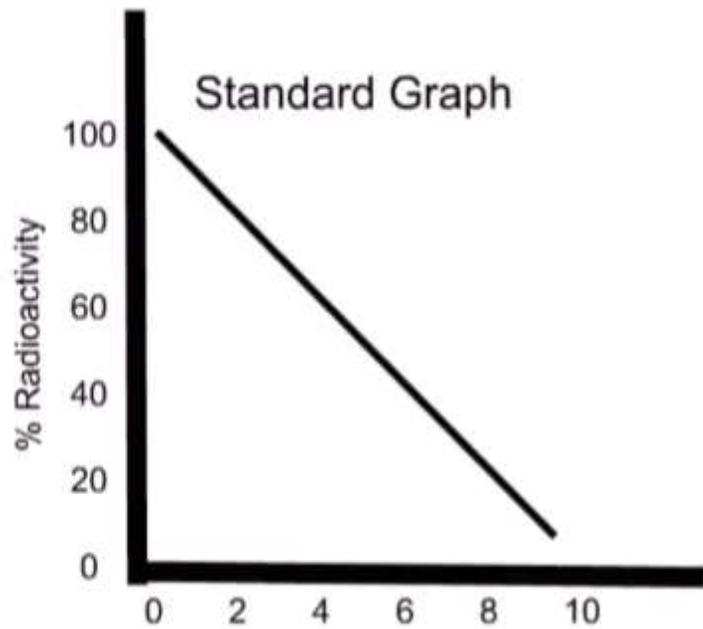
|   |     |
|---|-----|
| 0 | 100 |
|---|-----|

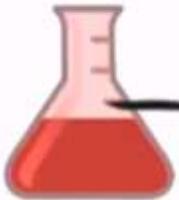
|   |    |
|---|----|
| 1 | 90 |
|---|----|

|   |   |
|---|---|
| - | - |
|---|---|

|   |     |
|---|-----|
| 0 | 100 |
|---|-----|

|   |     |
|---|-----|
| 0 | 100 |
|---|-----|



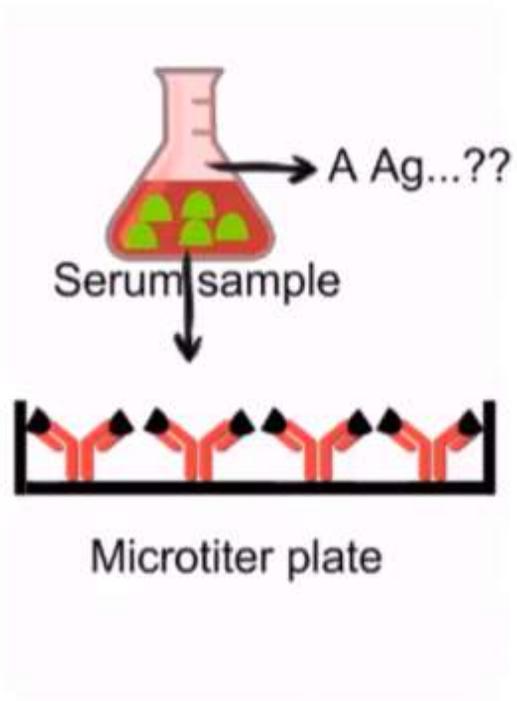


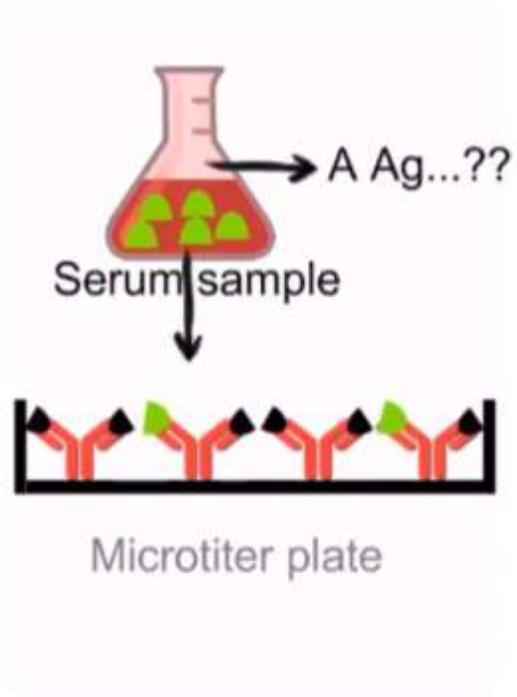
A Ag...??

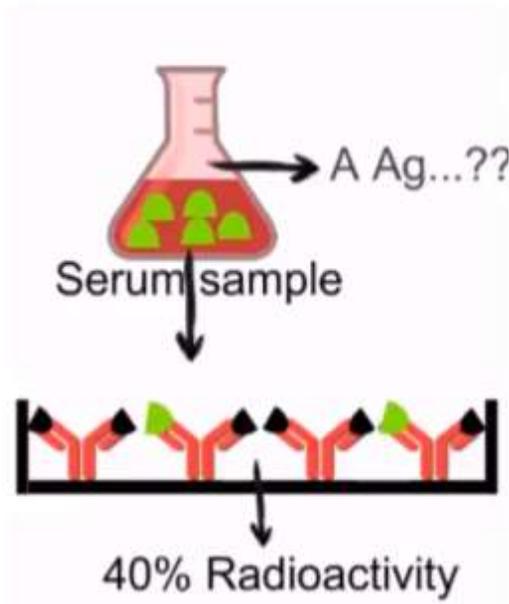
Serum sample



Microtiter plate







# RIA- Principle:



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The proportion of  $\text{Ab-Ag}^*$  and  $\text{Ab-Ag}$  at equilibrium will be equal to the original proportion of  $\text{Ag}^*$  to  $\text{Ag}$

# Disadvantages of RIA

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- 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

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- 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

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

# Direct ELISA

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Matrix or Surface

# Direct ELISA

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## Advantages:

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

# Direct ELISA

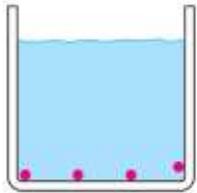
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## Disadvantages:

- 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

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Antigen-coated well



# Indirect ELISA - Advantages

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- 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

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- 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.

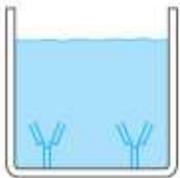
# Sandwich ELISA

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# Sandwich ELISA

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Monoclonal antibody-coated well



# Sandwich ELISA

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- 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

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- 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.

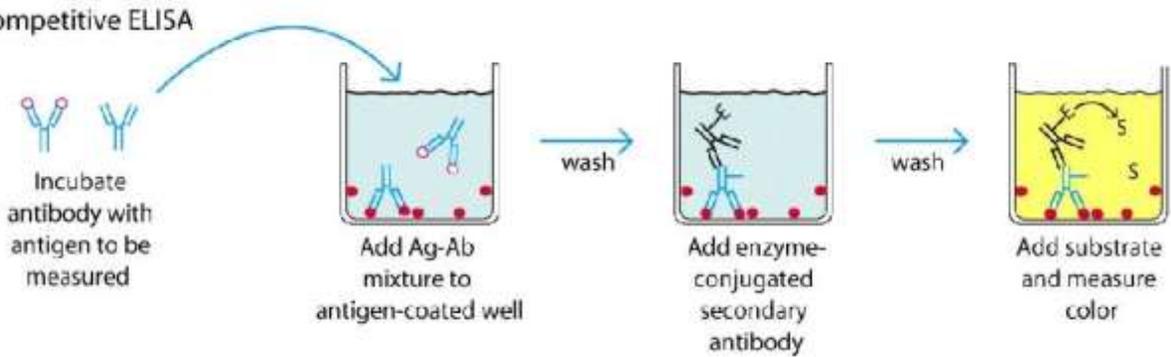
# Competitive ELISA

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- 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



The diagram illustrates the four steps of a competitive ELISA. Step 1: Two Y-shaped antibodies are shown, one with a red dot (antigen) on its tip. Step 2: A well with red dots (antigen) on the bottom contains a mixture of the antibodies and the antigen. Step 3: After washing, the well contains the antigen-coated antibodies and the enzyme-conjugated secondary antibodies. Step 4: After a second wash, the well contains the antigen-coated antibodies and the enzyme-conjugated secondary antibodies, with a yellow substrate being converted to a yellow color.

Incubate antibody with antigen to be measured

Add Ag-Ab mixture to antigen-coated well

wash

Add enzyme-conjugated secondary antibody

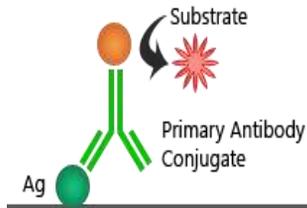
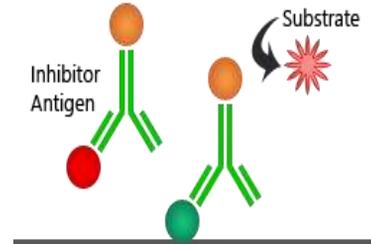
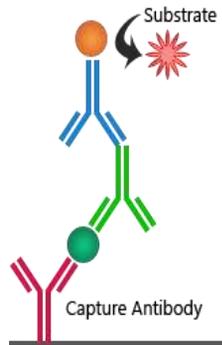
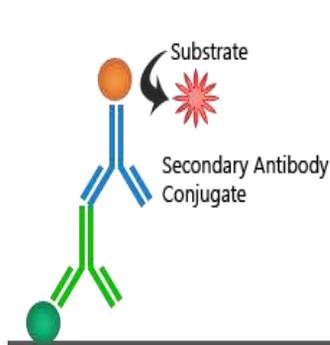
wash

Add substrate and measure color

# Comparison of the 4 types of ELISA

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

# Identify the type of ELISA:



# Which ELISA will you use?

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

# Steps of ELISA

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

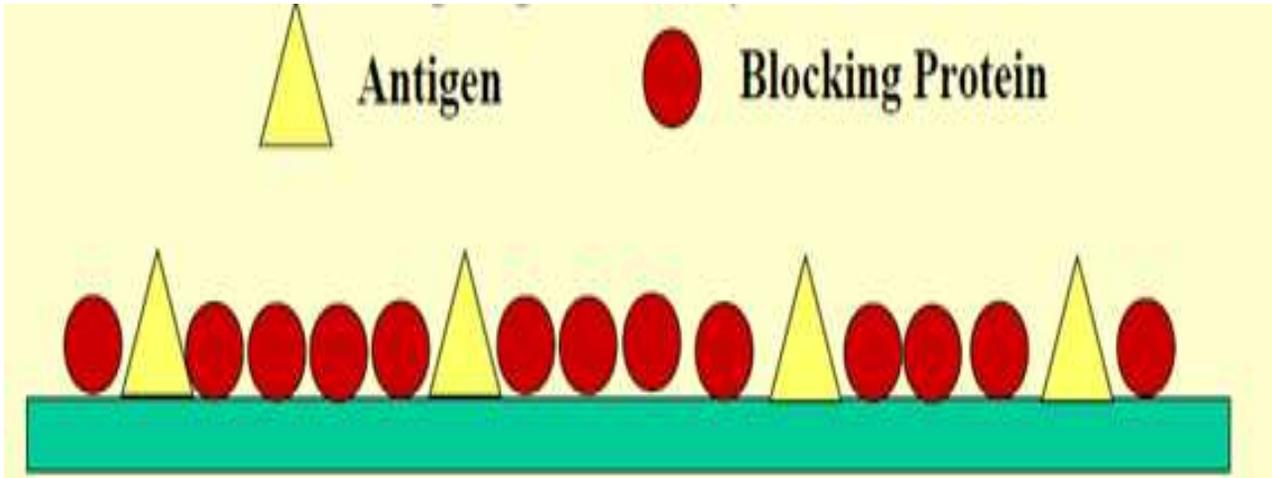
# Coating

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# Blocking

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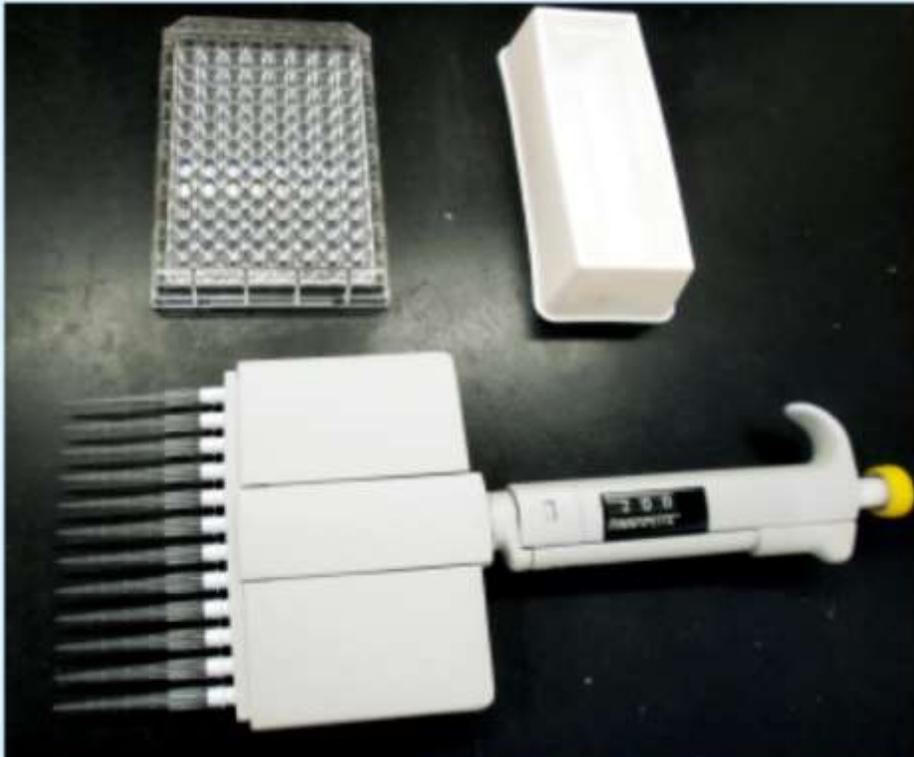
# Blocking agents

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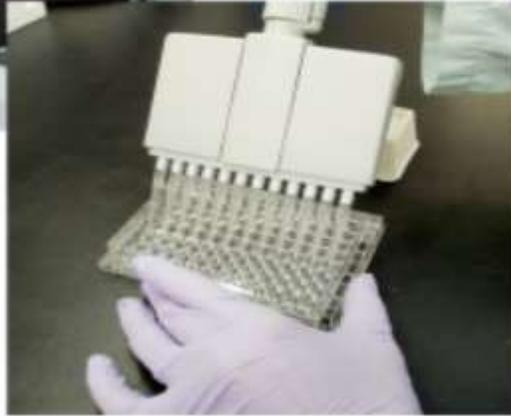
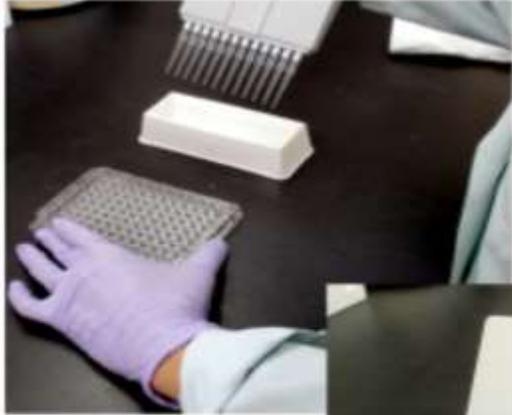
- 1% Bovine serum albumin (BSA)
- Serum
- Non-fat dry milk (NFM)
- Casein or Gelatin in PBS

96-well plate ▼

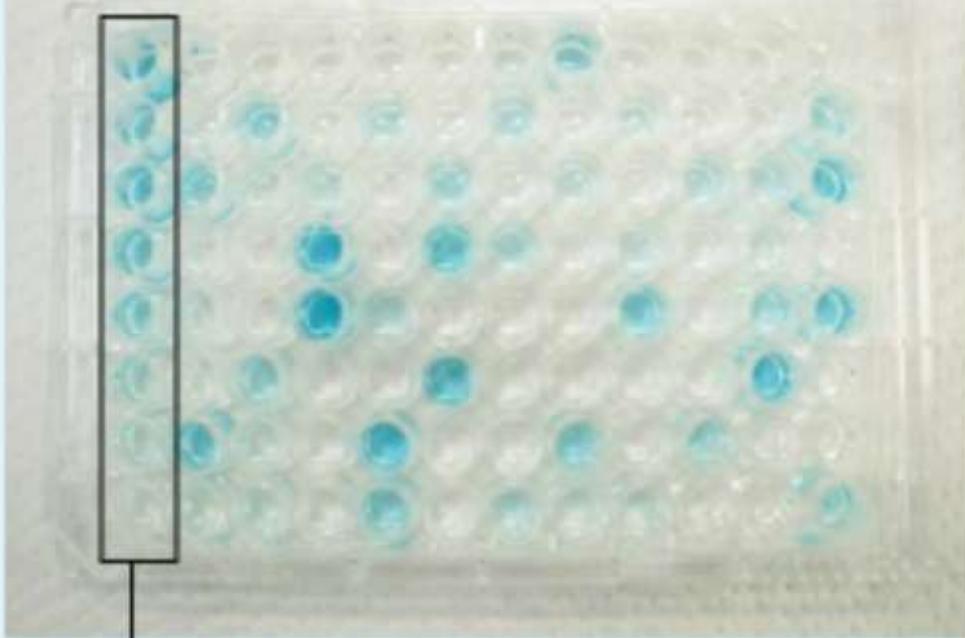
▼ Reservoir



▲ 12-Channel pipette

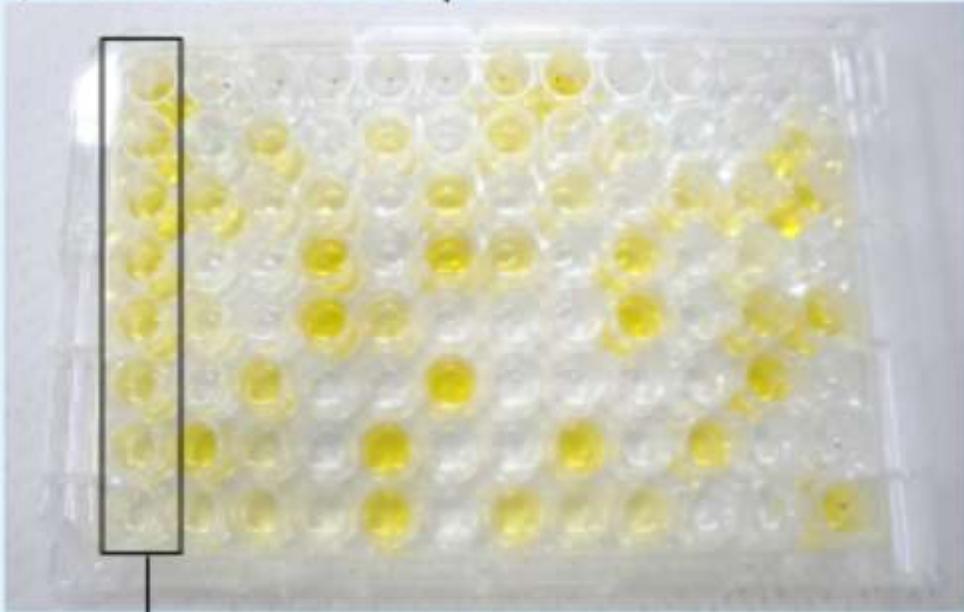


10 minutes after the addition of the substrate



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

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- 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.

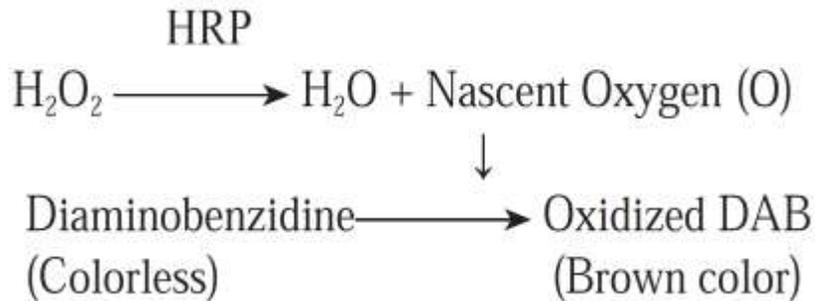
# Commonly used enzymes

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- Horseradish peroxidase
- Alkaline phosphatase

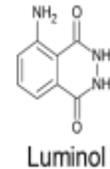
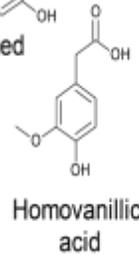
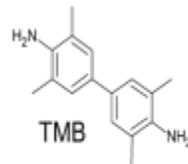
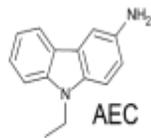
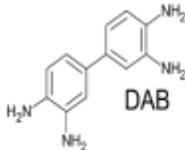
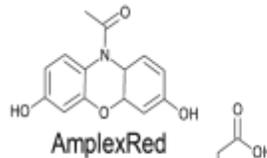
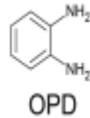
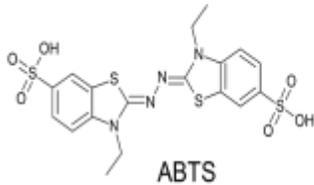
# Horse-radish peroxidase

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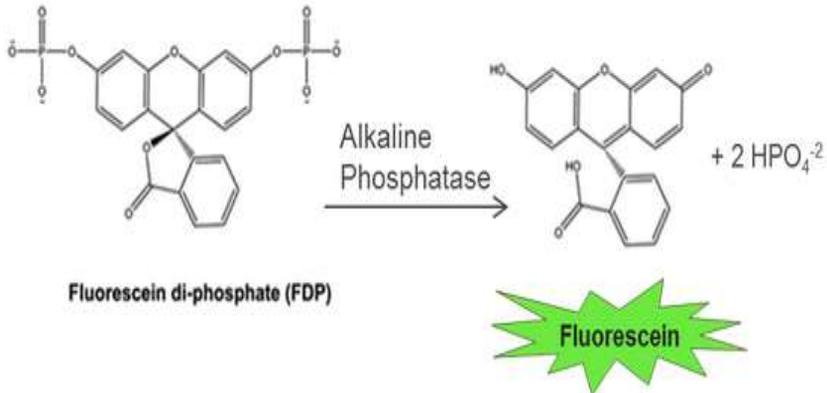
# Chromogenic Substrate

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# Alkaline phosphatase

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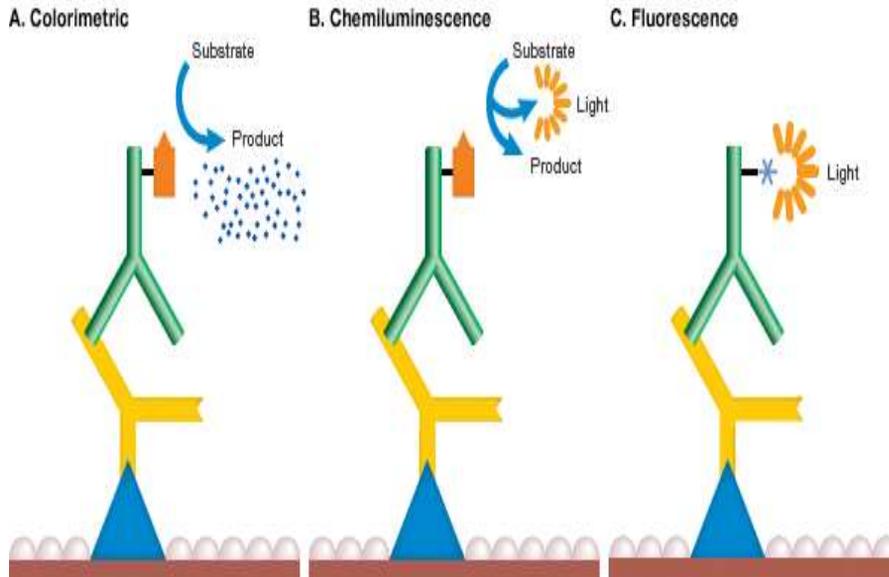


# ALP chromogenic substrate

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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

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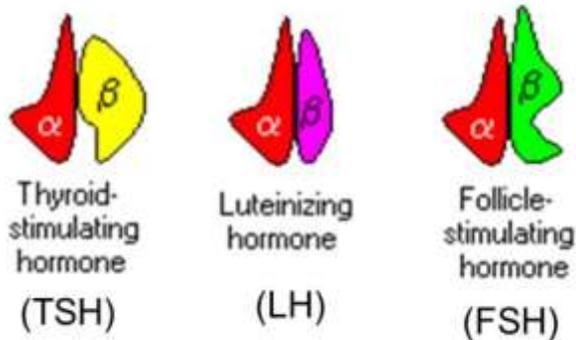
# Human chorionic gonadotrophin

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- 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  $\alpha$  subunit.
- Chromosome 19 contains genes for beta subunit.

# Alpha subunit is shared

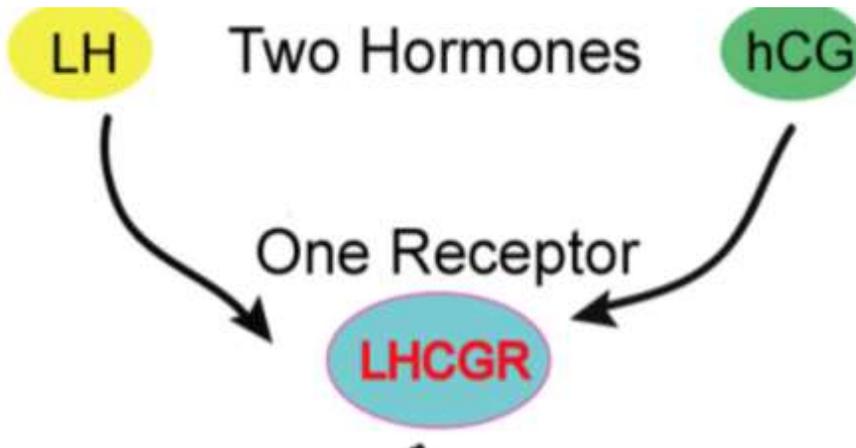
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Alpha subunit is shared by glycoprotein hormones (TSH, LH [luteinizing hormone], FSH, and hCG).

# hCG receptor

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# hCG and pregnancy

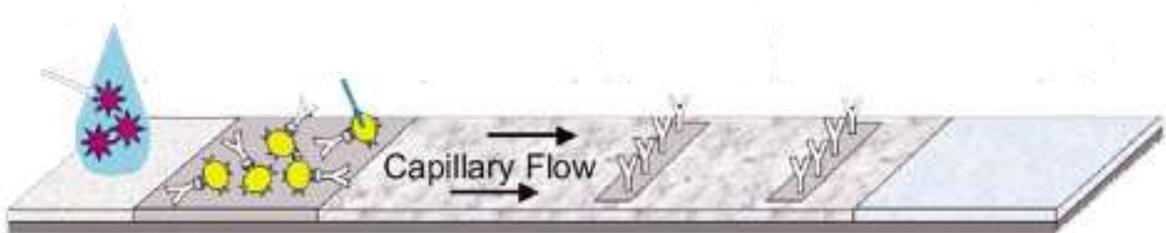
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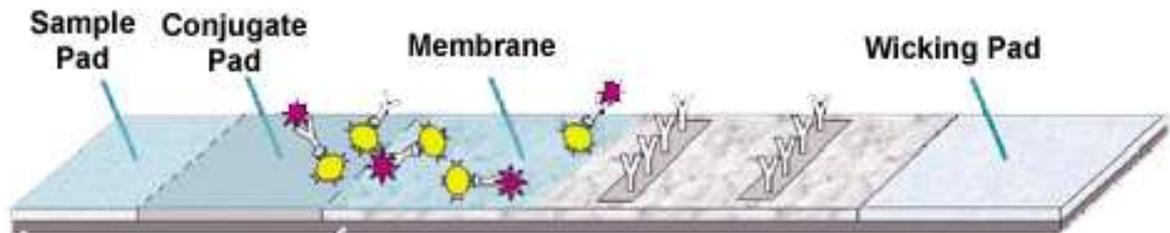
- Synthesis of hCG $\beta$  peaks at about 8 to 10 weeks of gestation.
- First-morning specimens are preferred for qualitative urine pregnancy tests because they are concentrated and contain abundant hCG.

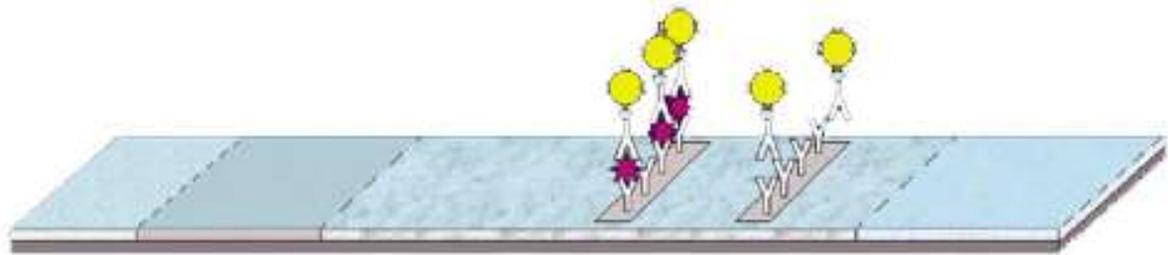
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# Lateral flow Immunoassay

# Lateral flow Immunoassay







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



# Interpret:



# Thank You!

