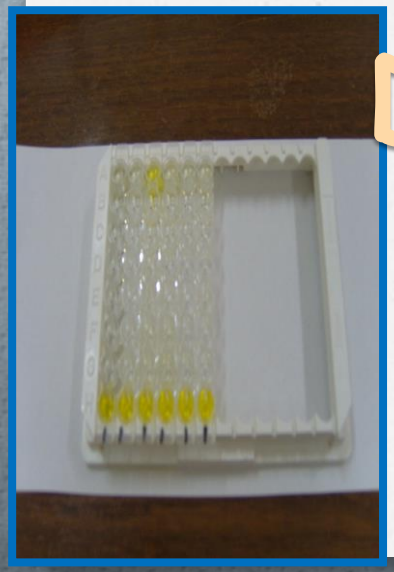
# Enzyme-Linked ImmunoSorbant Assay (ELISA)


By

Dr/ Rania Ewida

Ass. Prof. of Milk Hygiene

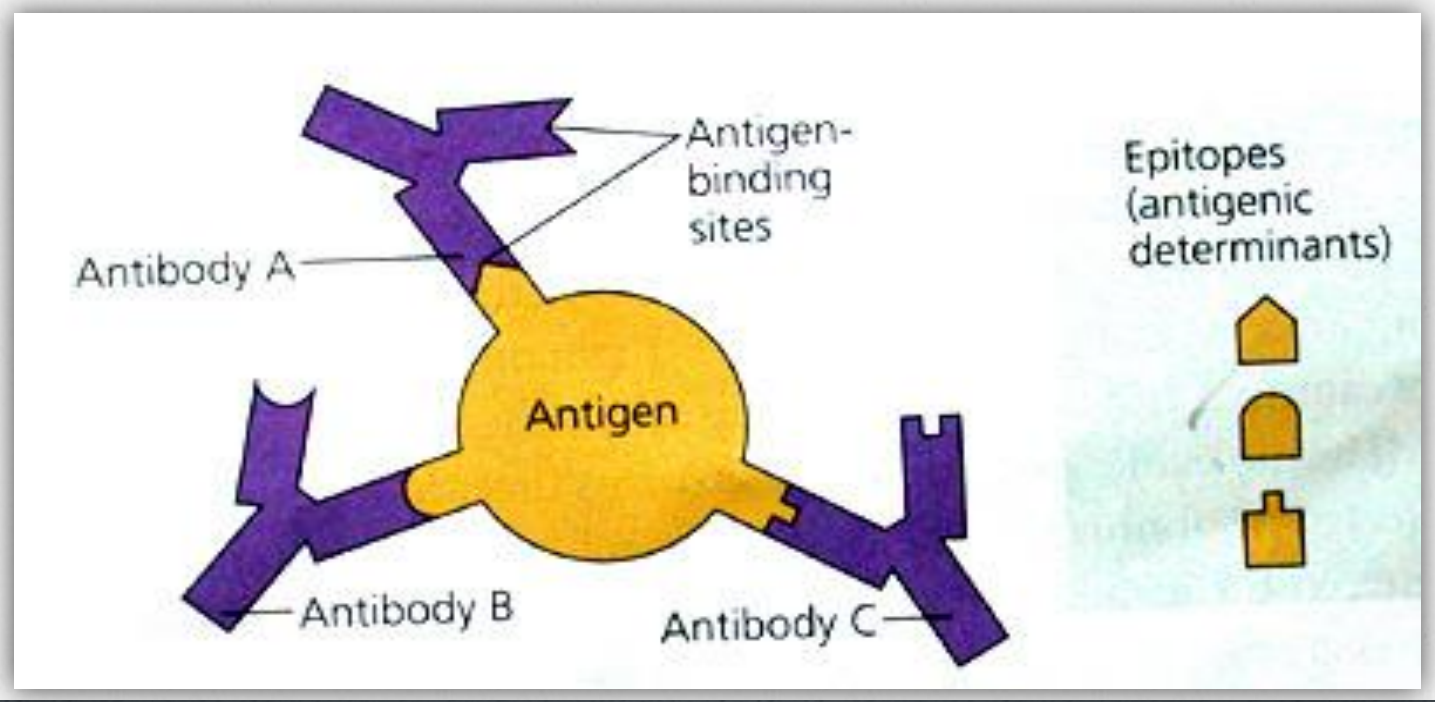



# ELISA

- ✓ **ELISA - an acronym for Enzyme-Linked ImmunoSorbent Assay.**
- ✓ **The ELISA assay is a widely used biochemical assay to detect in a sample the presence of and quantity of proteins, such as hormones and antibodies and bacteria or viruses.**
- ✓ **One can determine how much antibody is present by starting with an antigen, or one can determine how much antigen or hormone is present by starting with an antibody.**

# ELISA

**Immunoassay are based on the strong & highly specific interaction occurring between antigens (Ag) & antibodies (Ab)**

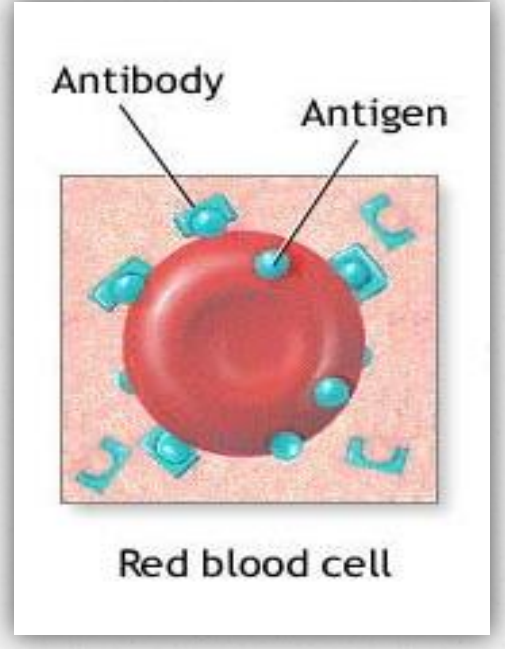


# What Are Antigens?

- Antigens are any foreign substance in the body.

- Such as:

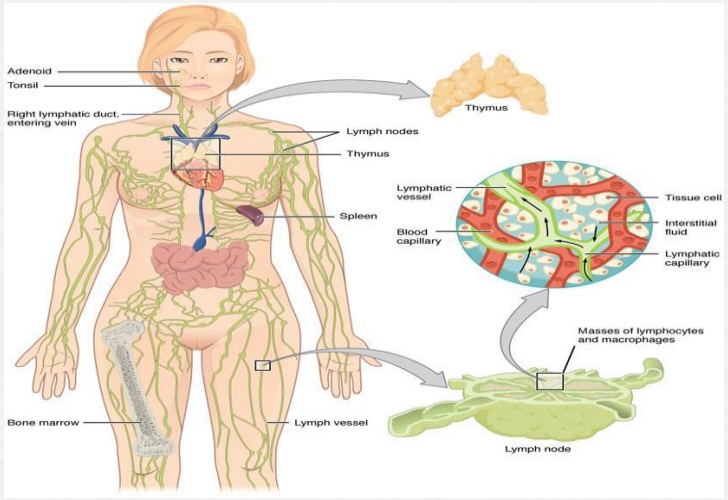
a. foreign proteins
b. viruses
c. environmental pollutants
d. bacteria and parasites
e. foreign transplanted tissue
f. cancerous cells




- Most antigen are proteins & its molecular weight is greater than 1000

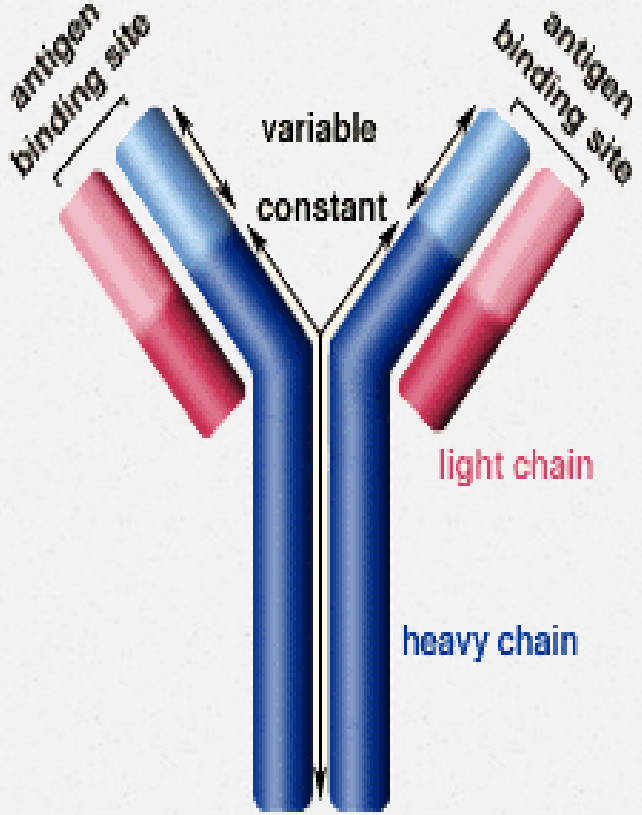
# What Are Antibodies and How Are They Produced?

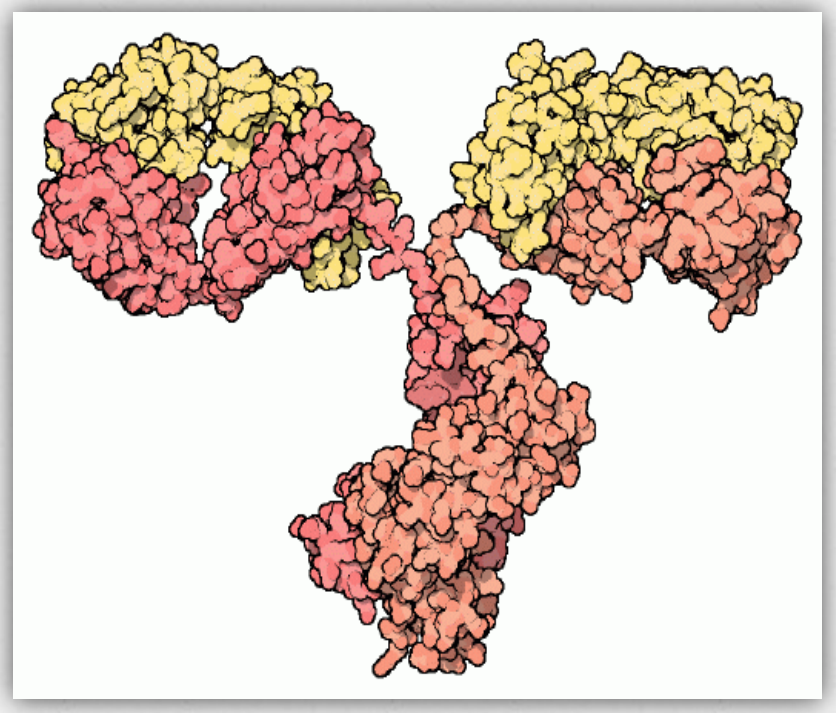
- ✓ Antibodies are large glycoprotein molecules produced by B-lymphocytes during the humoral immune response to antigens introduced into the body.
- ✓ B Lymphocytes Mature in the Bone Marrow and T Lymphocytes Mature in the Thymus Gland



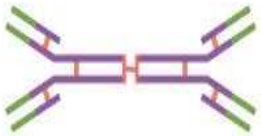
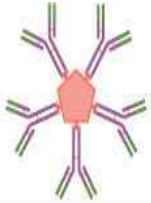
# What Are Antibodies and How Are They Produced?

## Structure of an antibody

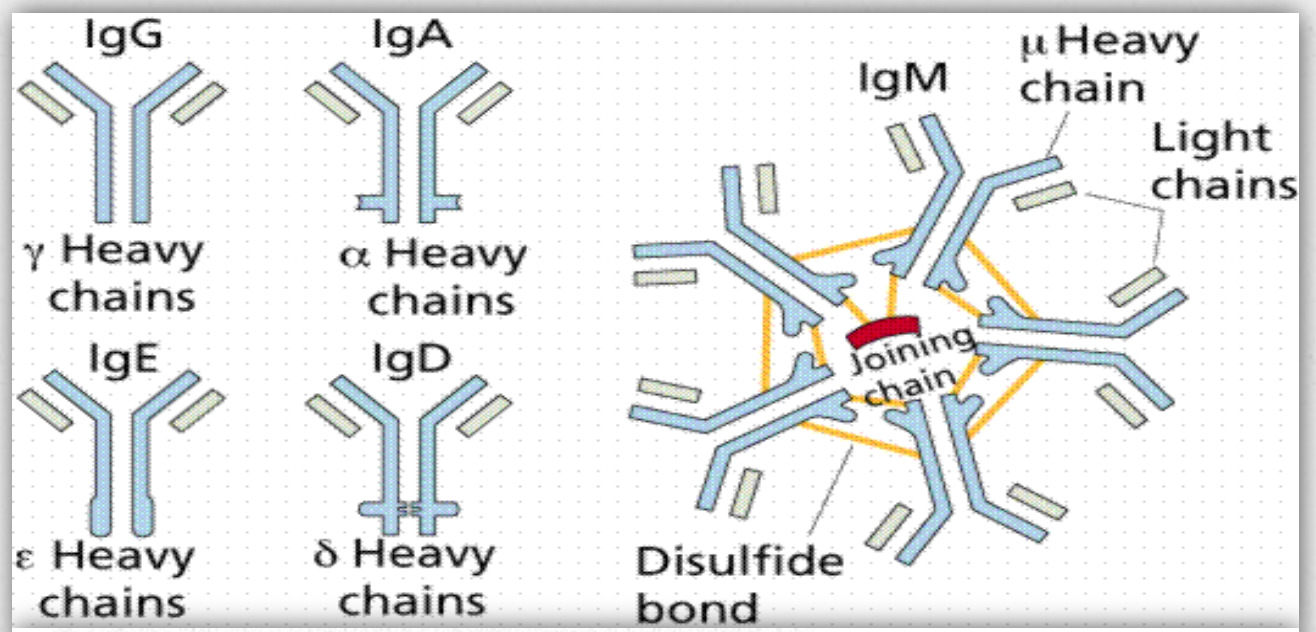


# Classes of antibodies

| Name | Properties | Structure |
|---|---|---|
| IgA | Found in mucous, saliva, tears, and breast milk. Protects against pathogens. |  |
| IgD | Part of the B cell receptor. Activates basophils and mast cells. |  |
| IgE | Protects against parasitic worms. Responsible for allergic reactions. |  |
| IgG | Secreted by plasma cells in the blood. Able to cross the placenta into the fetus. |  |
| IgM | May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity. |  |

# Classes of antibodies



# History of ELISA

## Identification:

**Is a method to determine the concentrations of a material in a solution**

**It was developed in 1970 & became rapidly accepted**

# ELISA Principle

As its name suggests, uses an enzyme system to show the specific combination of an antigen with its antibody

## The enzyme system consists of:

*. An enzyme which is labeled, or linked, to a specific antibody or antigen

*. A substrate which is added after the antigen antibody reaction

This substrate is acted on by the enzyme attached to the antigen antibody complexes, to give a color change

# ELISA Principle

**The intensity of the color gives an indication of the amount of bound antigen or antibody**

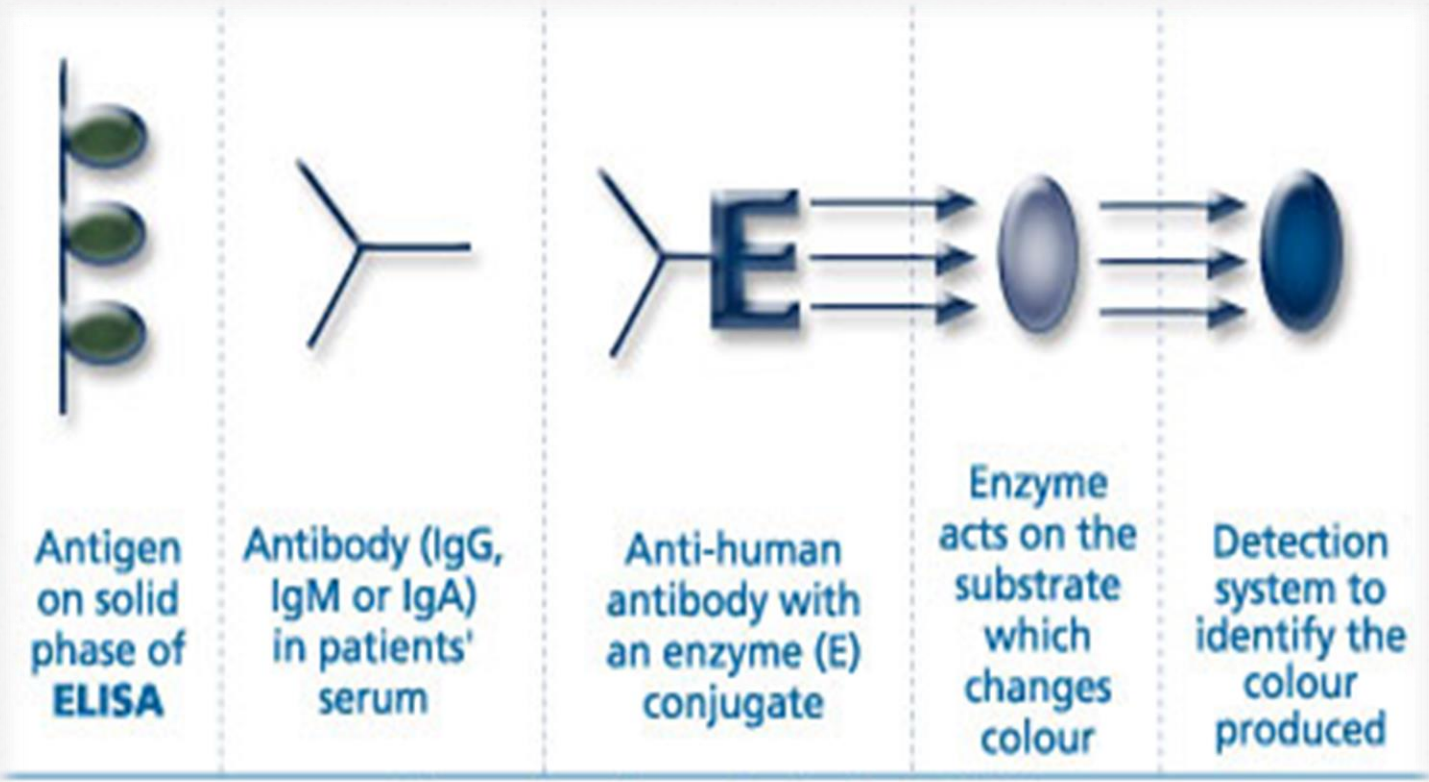
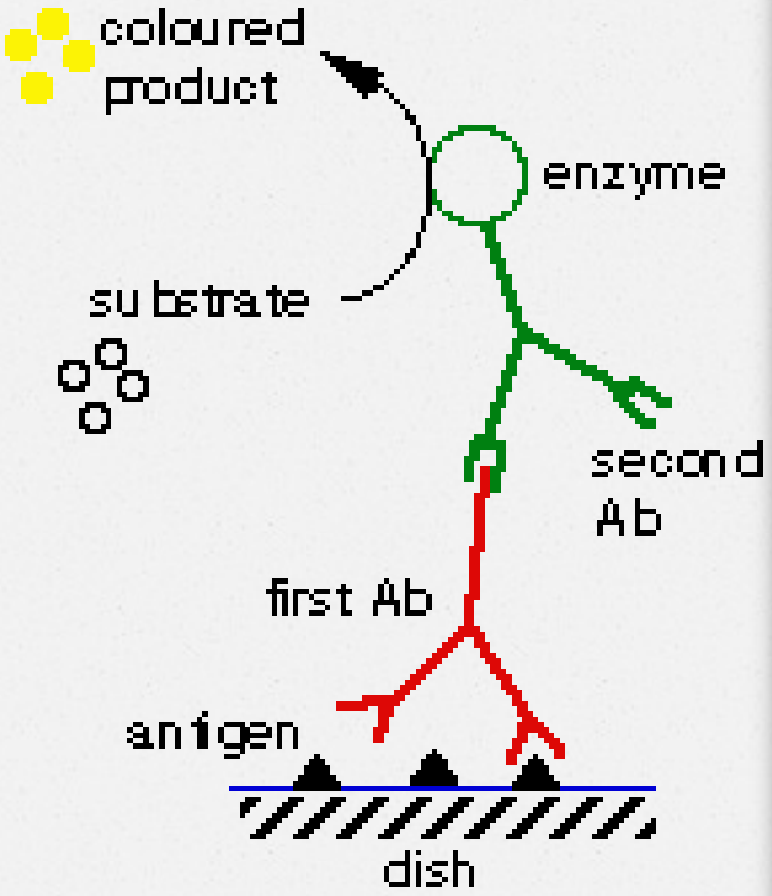
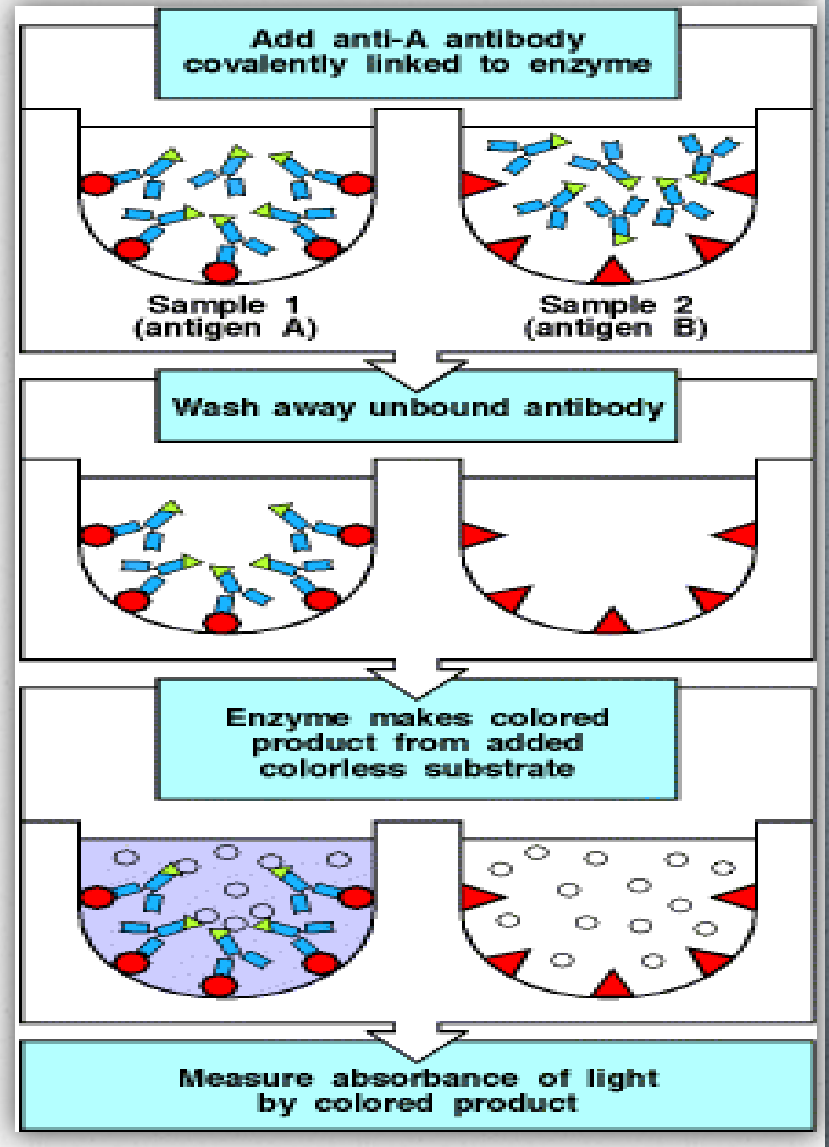


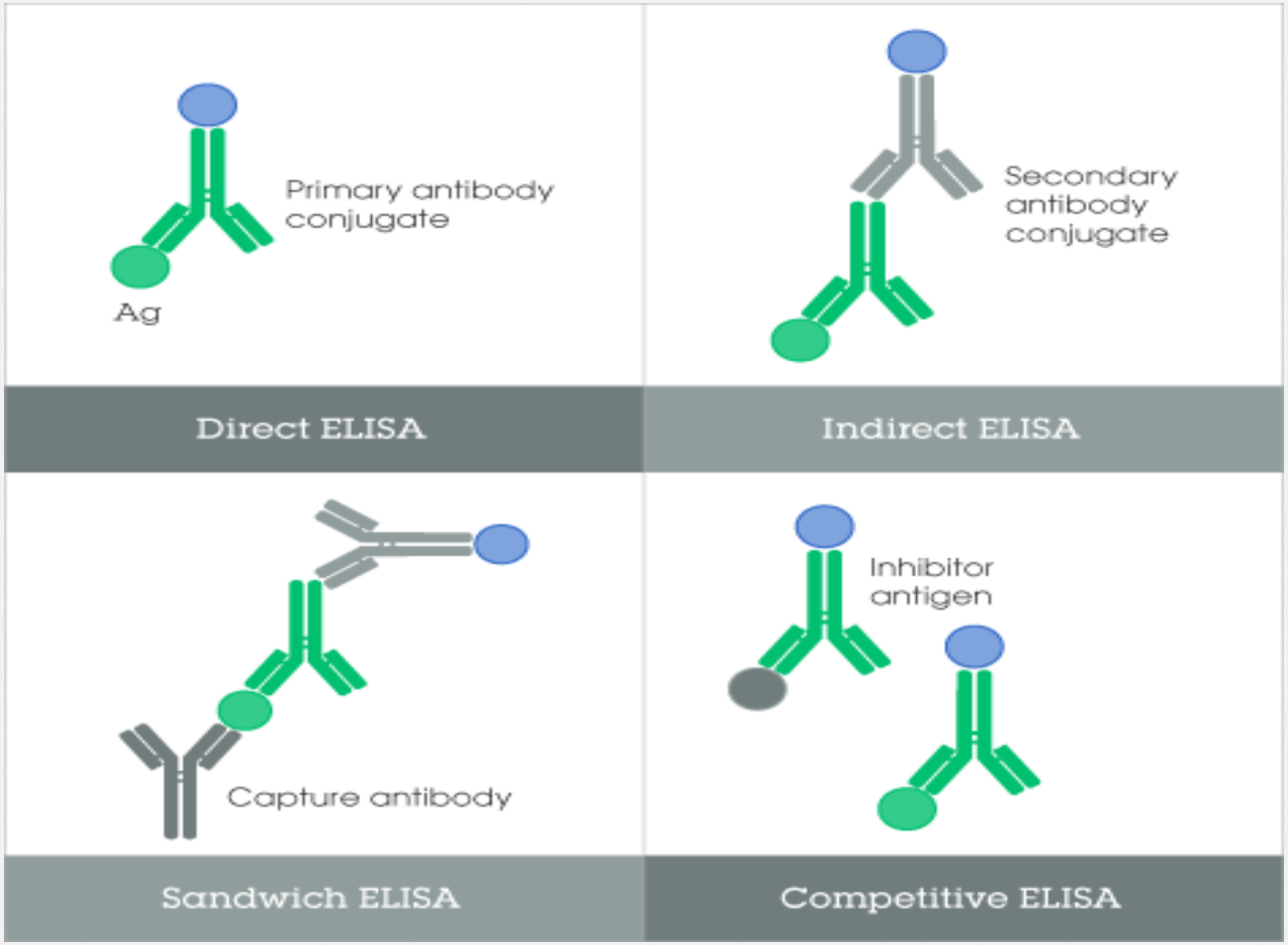

*Figure showing principle of ELISA*

# ELISA Principle

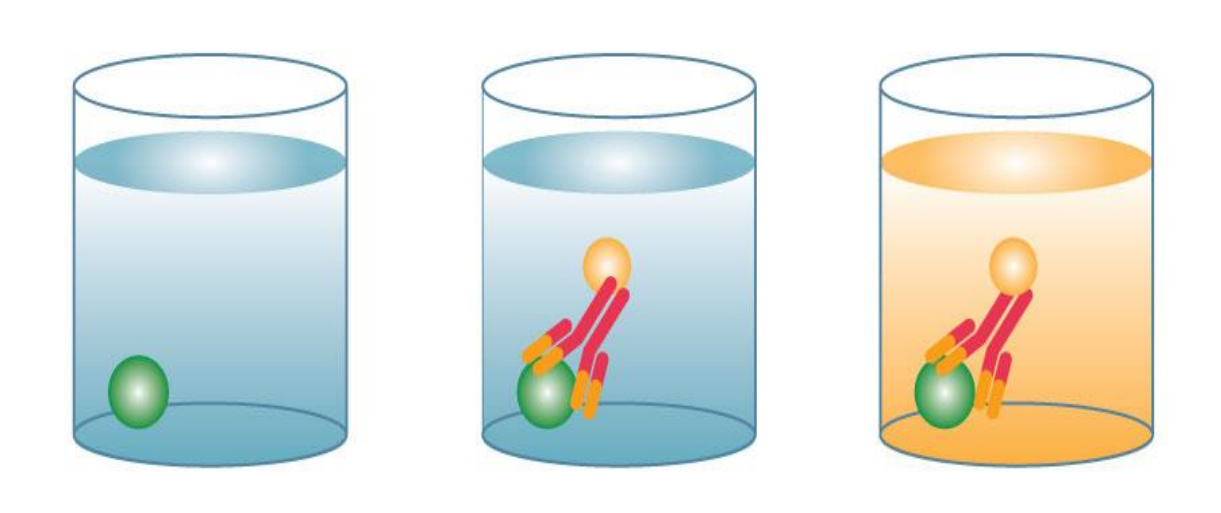


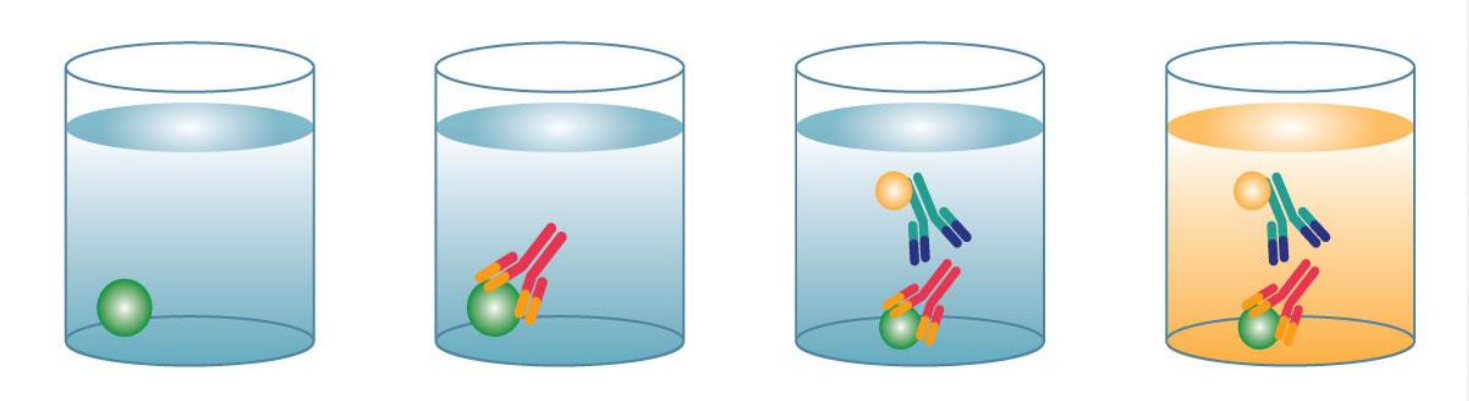
# Types of ELISA Methods



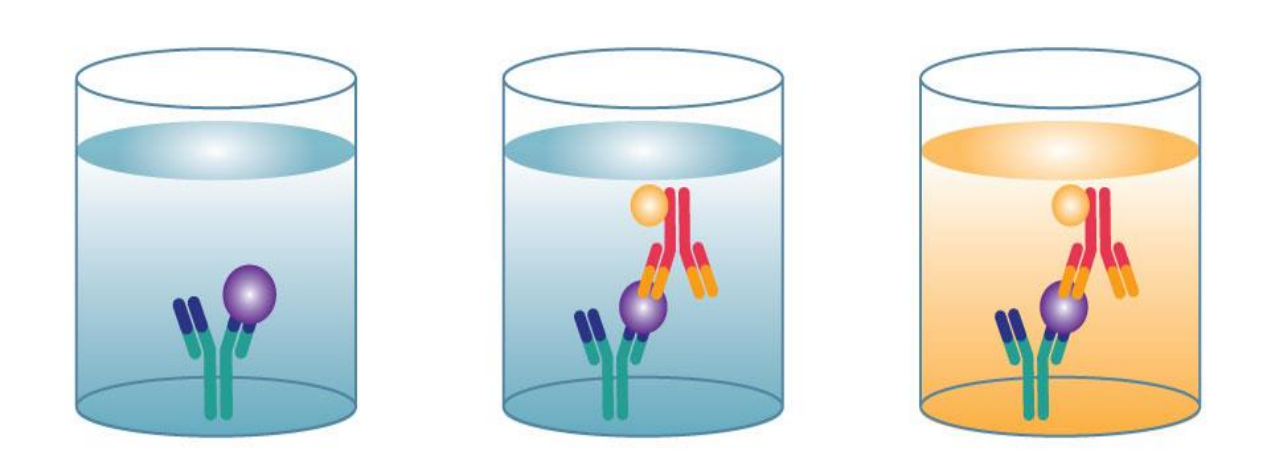
In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample. Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA.



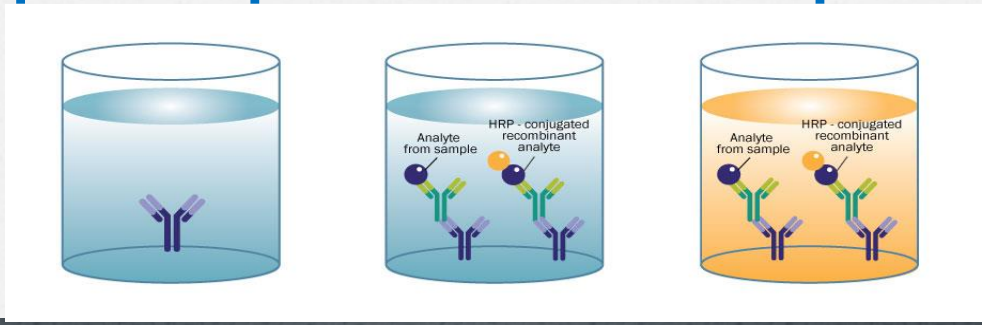
An indirect ELISA is similar to a direct ELISA in that an antigen is immobilized on a plate. First, an unconjugated primary detection antibody is added and binds to the specific antigen. A conjugated secondary antibody directed against the host species of the primary antibody is then added. Substrate then produces a signal proportional to the amount of antigen bound in the well.



Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated-detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample. Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.



Competitive ELISAs are commonly used for small molecules of protein. Similar to a sandwich ELISA, a capture antibody is coated on a microplate. Instead of using a conjugated detection antibody, a conjugated antigen is used to complete for binding with the antigen present in the sample. The more antigen present in the sample, the less conjugated antigen will bind to the capture antibody. Substrate is added and the signal produced is inversely proportional to the amount of protein present in the sample.



# The Indirect ELISA Method

a) **Binding Known Antigen** - coated the wells of a microtiter plate.

b) **Blocking** - by a concentrated solution of non-interacting protein, like casein or bovine serum albumin, to block other proteins in the test sample from adhering.

c) **Washing** – Rinse to remove any unbound.

d) **Adding Test Sample Primary Antibody** - The test sample of serum containing the primary antibodies

e) **Washing** – Rinse to remove any antibodies that did not bind to the known antigen.

# The Indirect ELISA Method

**f) <u>Adding Enzyme-linked Secondary Antibody</u>** - An enzyme-linked secondary antibody is added next to bind to the test sample antibodies. The enzyme on the secondary antibodies are proteins, such as horse radish peroxidase or alkaline phosphatase.

**g) <u>Washing</u>** – Rinse to remove any secondary antibodies that did not bind to the primary antibody.

**h) Adding Substrate** - A substrate is then applied which is converted by the enzyme to give a color or fluorescence or electrochemical signal. In the presence of horse radish peroxidase, ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid] turns green, OPD (o-phenylenediamine dihydrochloride) turns orange, and TMB (3,3',5,5'-tetramethylbenzidine) turns blue. Addition of stop solution, pNPP (p-Nitrophenyl Phosphate) turns yellow.

**i) <u>Reading Results</u>** - By using a spectrophotometer, the results can be read and recorded.

# The Indirect ELISA Method


# Advantages of ELISA

- **Sensitive:** nanogram levels or lower
- Minimal reagents
- Qualitative & Quantitative
  - Qualitative → eg. HIV testing
  - quantitative assays → Eg Ther. Drug Monitoring

# Advantages of ELISA

- **Greater scope** : Wells can be coated with Antigens OR Antibodies
- Suitable for automation → high speed
- NO radiation hazards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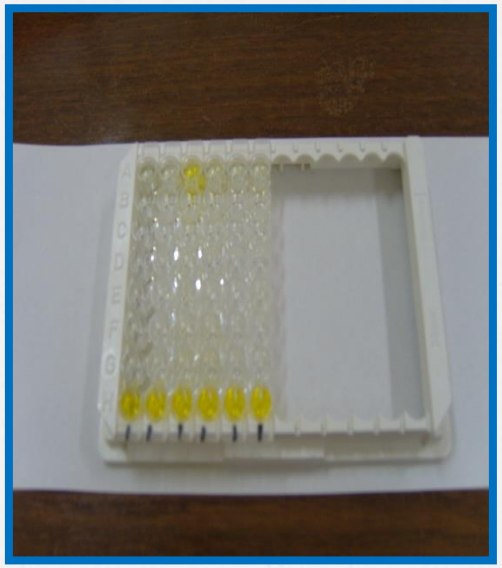
# The Different Application of ELISA

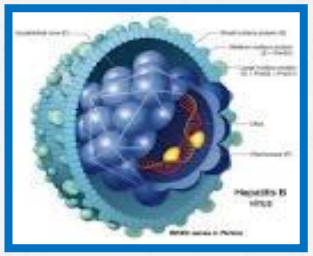


# Screening Donated Blood

- ✓ HIV
- ✓ Hepatitis C
- ✓ Hepatitis B

# Detection of Infectious Agents

- ✓ Bacteria e.g. *Campylobacter*
- ✓ Fungi
- ✓ Virus e.g. HBV
- ✓ Parasites e.g. *Leishmania*



## Measuring toxins in contaminated food

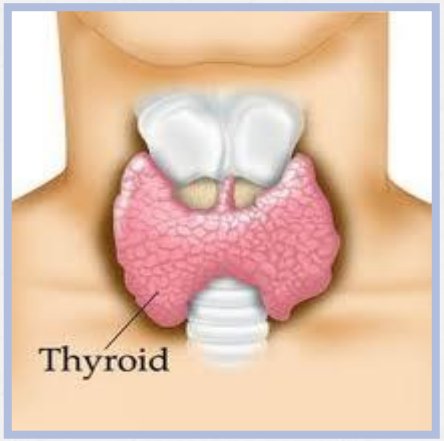
- ✓ Toxins of bacteria e.g. *Staph. aureus*
- ✓ Toxins of fungi e.g . Aflatoxins
- ✓ Environmental toxic substances e.g . Cadmium

## Measuring food allergens in food industry

- ✓ e.g. Milk, peanuts & eggs

# Measuring Hormone Levels

- ✓T3 & T4 (for thyroid function)
- ✓ HCG (as a test for pregnancy)
- ✓LH (determining the time of ovulation)



## Measuring Proteins Levels

- ✓ Serum proteins e.g. albumin & immunoglobulin

## Measuring Minerals Levels

- ✓ e.g. Ca

## Measuring Vitamins Levels

- ✓ e.g. Vit. A, Vit. B 12 & Vit. D

## Detection of Tumor Markers

- ✓ Carcinoembryonic antigen (CEA)
- ✓ α-fetoprotein (AFP)

## Detection of Diseases Markers

- ✓ Detection rheumatoid factors which has important role in diagnosis rheumatoid arthritis

# Detection of Drug Markers

- ✓ Drug residue in food e.g. Sulfamethazine
- ✓ Detection illicit drug e.g. Cocaine opiates
- ✓ Drug discovery by screening the compounds
- ✓ Toxicology as a rapid presumptive screen for drugs

# New born Screening


✓ **e.g.** Sickle cell anaemia

# Genetically Modified Organisms(GMO)

✓ Qualitative and quantitative detection for targeted protein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