MBRU

ENZME LINKED IMMUNOSORBENT ASSAY (ELISA)

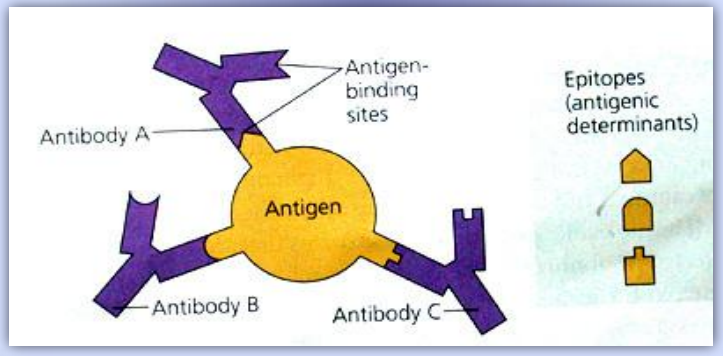
BY

PROF. DR. ASMAA HUSSEIN

DIRECTOR OF THE MOLECULAR BIOLOGY RESEARCH UNIT

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

Ag + Ab → AgAb



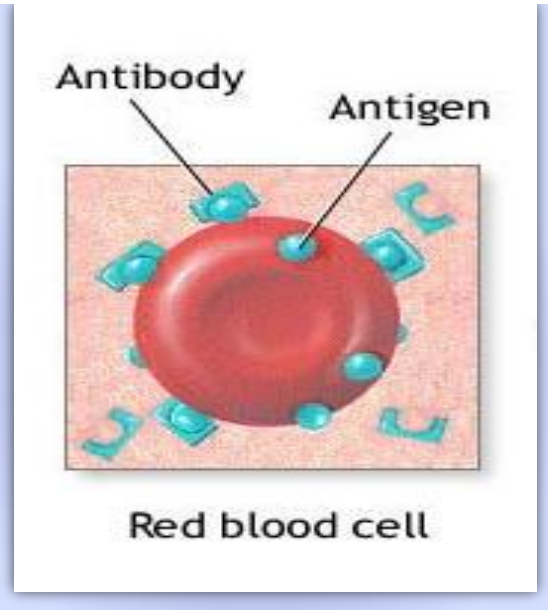
Antigen (Ag) :

Is a substance that when introduced into the body causes the production of antibodies

These antibodies will combine specifically with the antigen that caused their production

(Ag) Antigen

- **Soluble substance**
- **Toxins**
- **Substance present on bacteria, virus, red cells, or other type of cells**

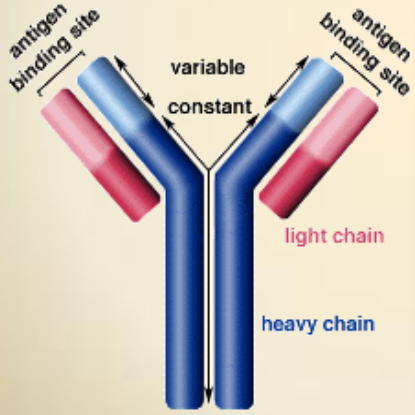


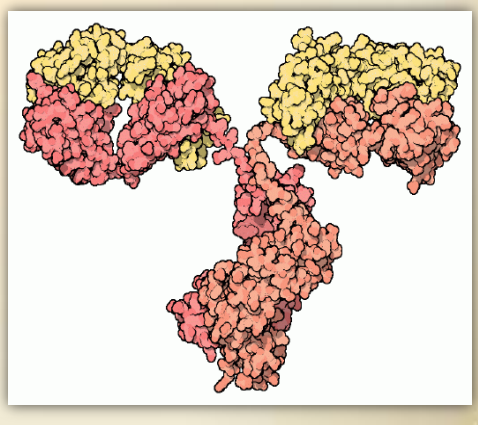
Most antigen are proteins & its molecular weight is greater than 1000

Antibodies (Ab):

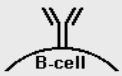
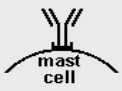
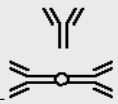
Is a protein produced by the immune system in response to the presence of an antigen

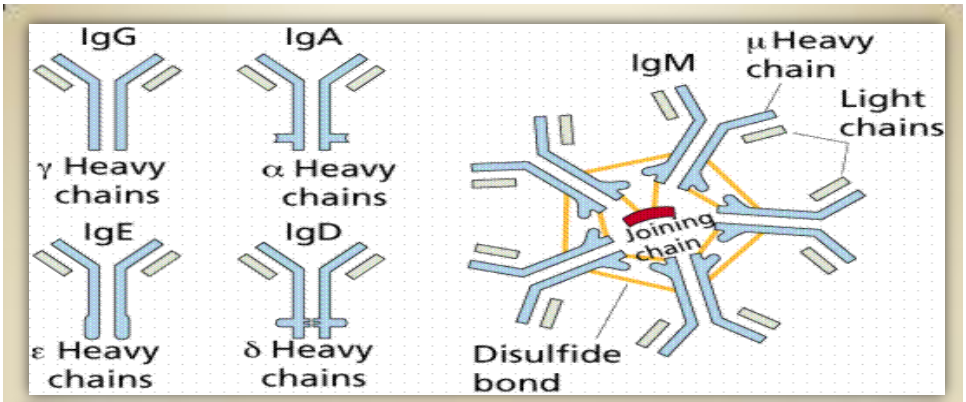
Structure of an antibody

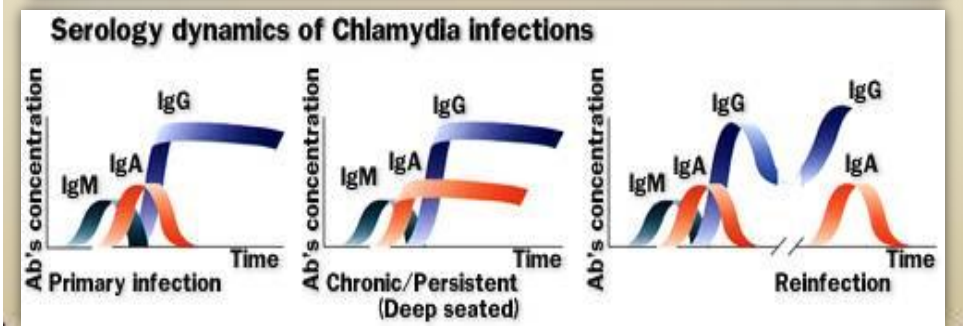




Classes of antibodies

Isotype	Structure	Placenta transfert	Activates complement	Additional features
IgM		No	Yes	First Ab in development and response
IgD		No	No	B-cell receptor
IgG		Yes	Yes	Involved in opsonization and ADCC. Four subclasses; IgG1, IgG2, IgG3, IgG4
IgE		No	No	Involved in allergic responses
IgA		No	No	Two subclasses; IgA1, IgA2. Also found as dimer (sIgA) in secretions.





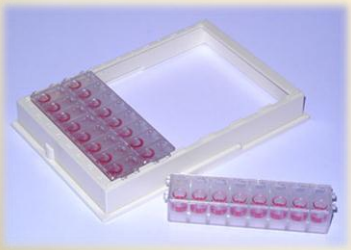
Characteristics of Human Immunoglobulins

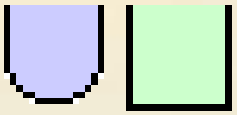
	IgG	IgA	IgM	IgD	IgE
Molecular weight	150,000	150,000-350,000	900,000	180,000	190,000
Sedimentation coefficient ($S_{20,w}$)	7	7(9-15)	19	7	8
Carbohydrate (approx. %)	3	7	12	12	12
Biological survival (plasma T-1/2 days)	21	6	5	3	2
Placental transfer	+	-	-	-	-
Activation of classical complement system	+	-	+	-	-
Serum concentration (mg per 100 ml)	1,100	250	100	3	.01

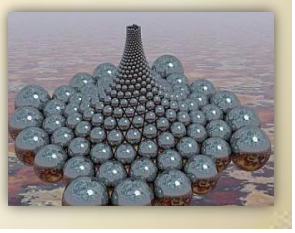
Immunoassay can be classified as homogeneous & heterogeneous

Heterogeneous:

One of the immunoreagents (either antigen or antibody) is bound to a solid phase (usually wells of a microtitre plate or magnetic microspheres)

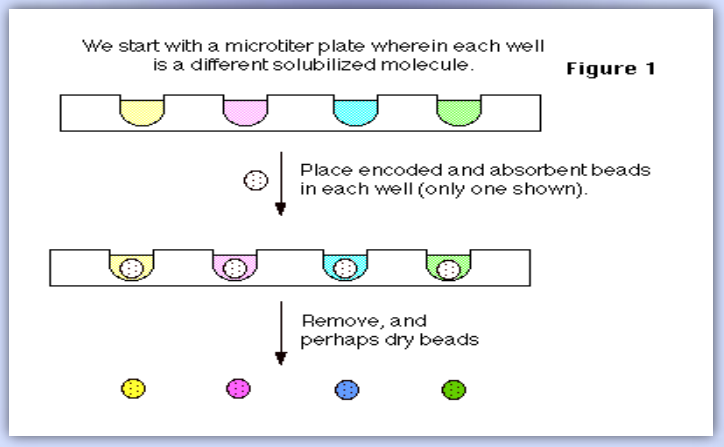






Homogeneous:

The antigen - antibody reaction occurs in a solution and there is no separation step of the tracer bound and free fractions before the measurement



Enzyme Linked Immunosorbent Assay

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

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

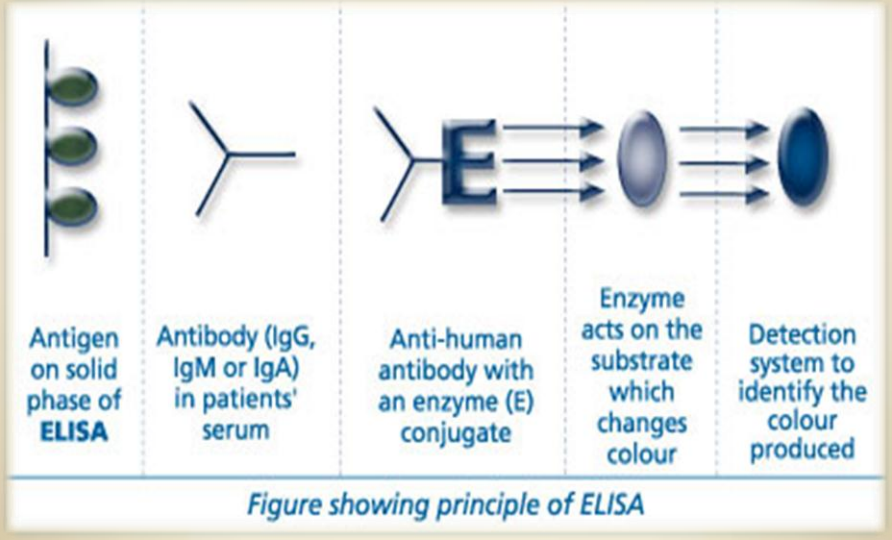


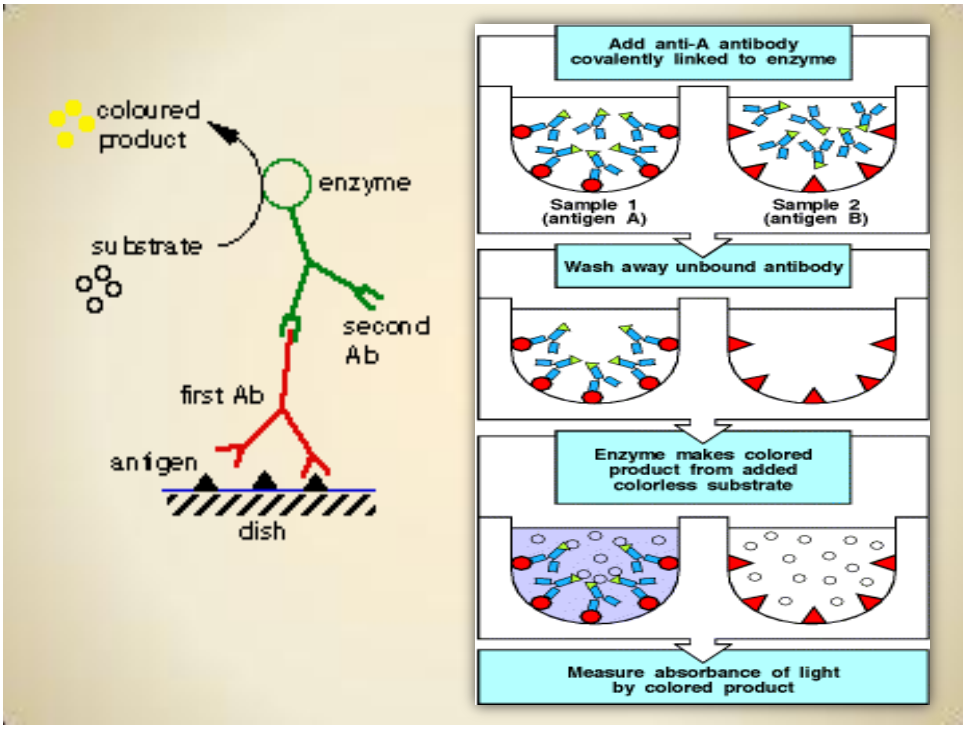
Figure showing principle of ELISA

Selecting enzyme label

- Horseradish peroxidase
- Calf intestine alkaline phosphatase
- E.coli – D - galactosidase

Selecting suitable substrate

- O-phenylenediamine dihydrichloride
- P-nitrophenyl phosphate



ELISA Types

1) Noncompetitive binding assay or Sandwich method:

A- Antigen measuring system [Titrewells coated with antibodies ; Enzyme labelled antibodies]

B- Antibody measuring system [Titrewells coated with antigens ; Enzyme labelled antiantibodies]

2) Competitive binding assay [Titrewells coated with antibodies ; Enzyme labelled antigens]

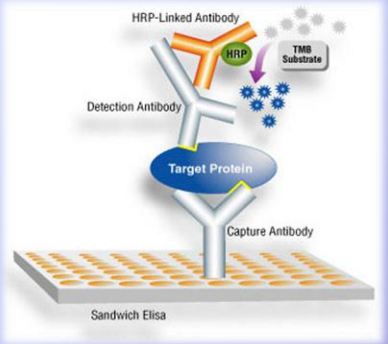
1)Noncompetitive or Sandwich Assay

A- Antigen measuring system

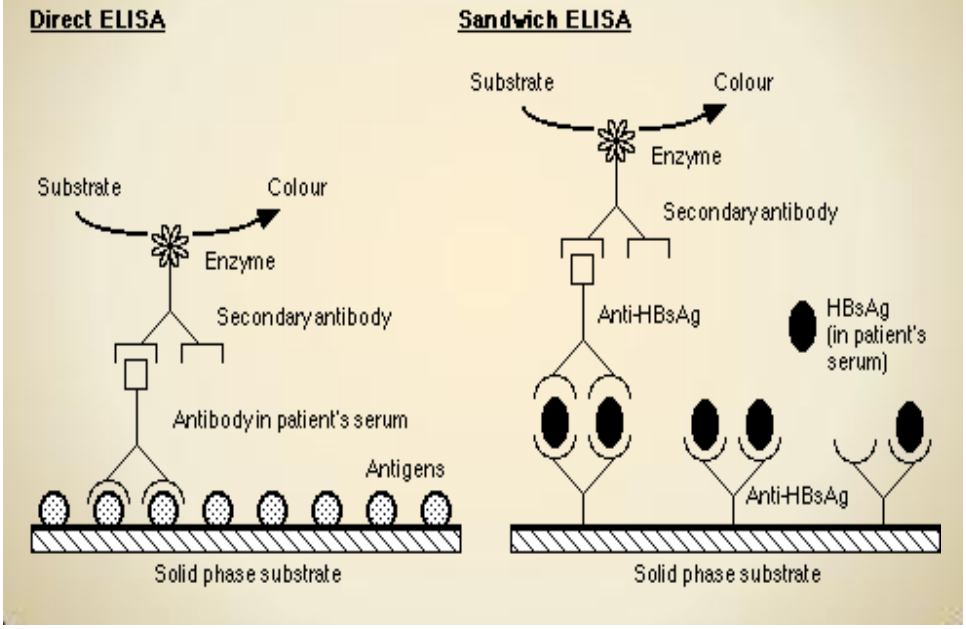
- ☑ Titre wells coated with suitable *antibody*
- ☑ Add patient sample containing *antigen*
- ☑ Incubate till antigen antibody reaction is complete
- ☑ Wash → remove unbound antigen
- ☑ Add Antibody labelled with Enzyme
- ☑ Incubate till antigen binds labelled antibody

- ☑ Wash → remove unbound labelled antibody
- ☑ Add substrate & incubate
- ☑ Enzyme + Substrate → Product → measure colour
- ☑ Colour proportional to antigen in patient sample

Elisa.mht



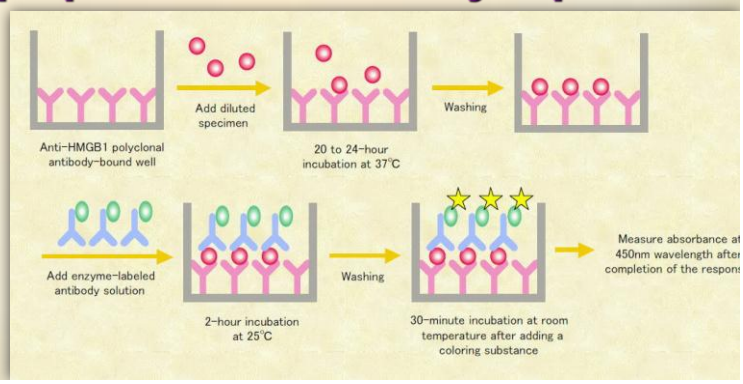
Sandwich Elisa



B- Antibody measuring system

- Titre wells coated with suitable antigen
- Add patient sample containing antibody
- Incubate till antigen antibody reaction is complete
- Wash→ remove unbound antibody
- Add Antiantibody labelled with Enzyme
- Incubate till labelled antiantibodies binds antigen-antibody complex

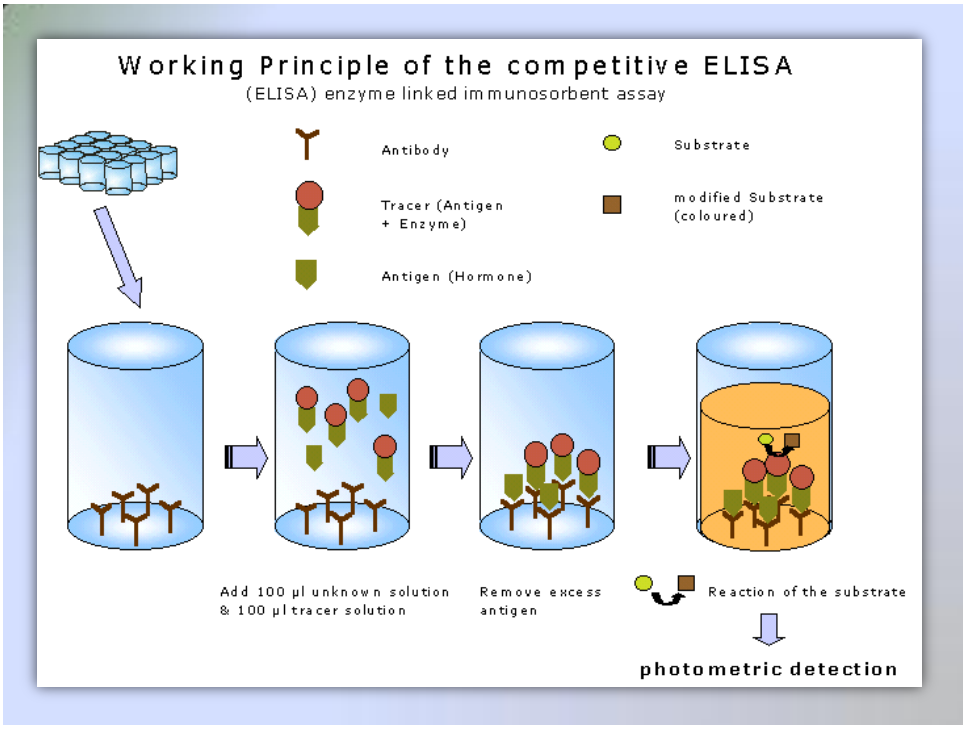
- Wash → remove unbound labelled antiantibody
- Add substrate & incubate
- Enzyme + Substrate → Product → measure colour
- Colour proportional to antibody in patient sample



2)Competitive binding assay

- Titrewells coated with antibodies
- Known quantities of patient sample containing antigen + antigen labelled with enzyme
- Incubate till antigen antibody reaction is complete
- Wash → remove unbound antigens
- Add substrate & incubate

- Enzyme + Substrate → Product → measure colour
- Colour inversely related to antigen in patient sample



Advantages of ELISA

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

- ***Greater scope*** : Wells can be coated with Antigens OR Antibodies
- Suitable for automation → high speed
- NO radiation hazards