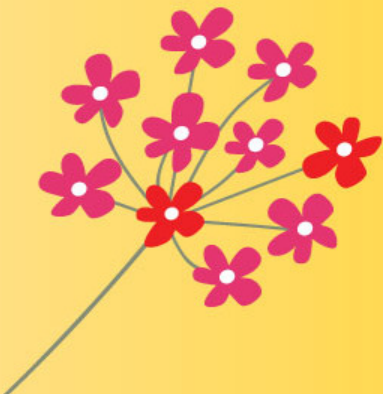


The background of the slide is a vibrant autumn-themed collage. It features several bright orange pumpkins of various sizes at the bottom, surrounded by a dense layer of colorful leaves in shades of orange, yellow, and red. The leaves are scattered throughout the frame, creating a rich, seasonal atmosphere. The text is centered in the upper half of the image.

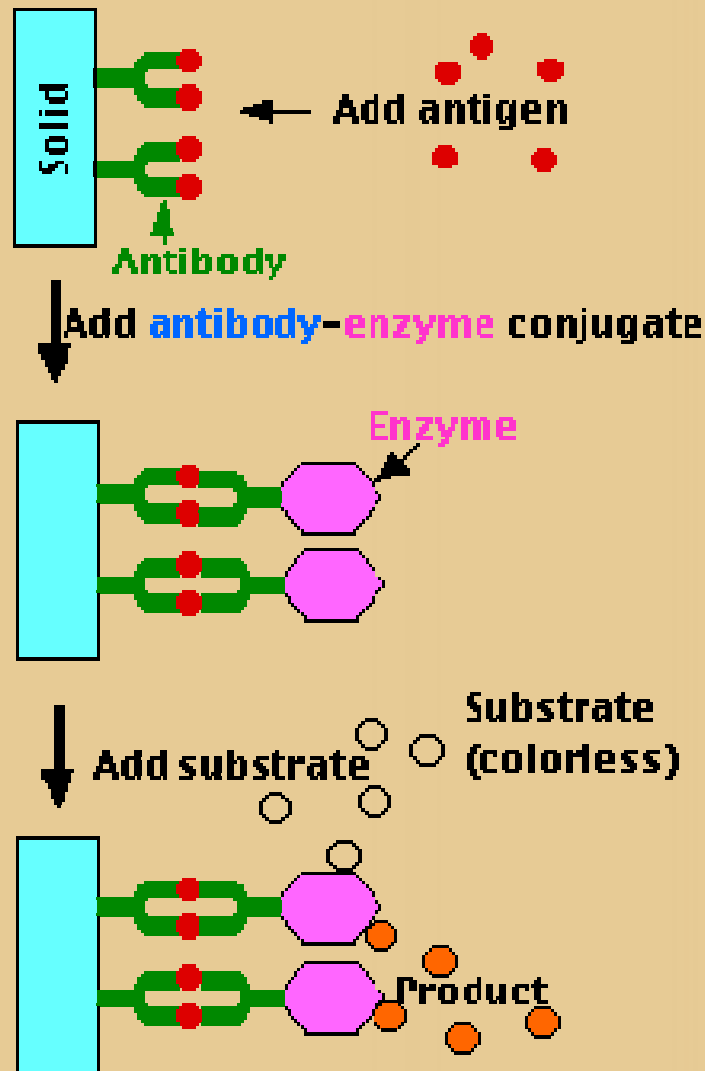
ELISA
Enzyme Linked
Immune Sorbent Assay

Introduccion

- ❑ Test are Specific and Sensitive
- ❑ It is the most common, widely used serological test for Ab or Ag detection.
- ❑ Ag or Ag are labelled by linking of enzyme.
- ❑ These test can be automated.
- ❑ It is method to determine the concentration of material.



Basic Principle of ELISA



ELISA Types

Direct (Sandwich)

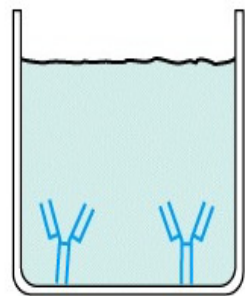
Indirect

Competitive



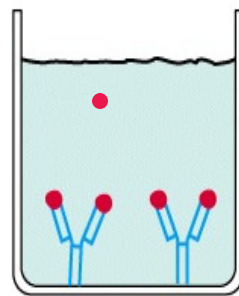
Direct method (Sandwich)

(Detection of Ag)



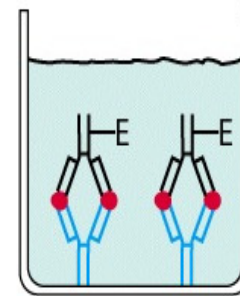
Antibody-coated well

wash →



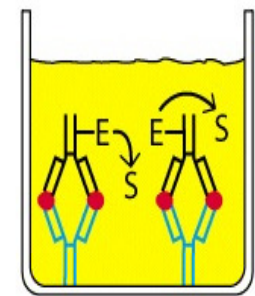
Add antigen to be measured

wash →



Add enzyme-conjugated secondary antibody

wash →



Add substrate and measure color



Enzyme

Substrate

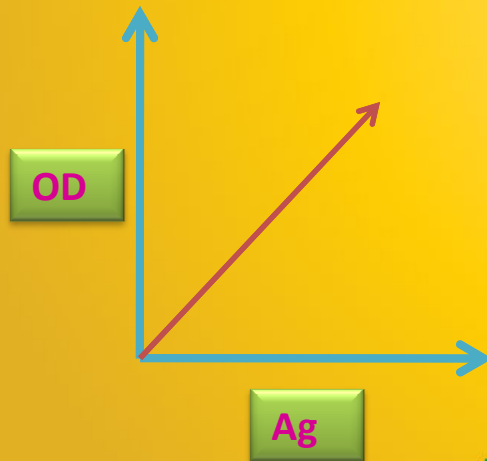
Peroxidase

H_2O_2

Alkaline phosphatase

P- Nitro phenyl Phosphate

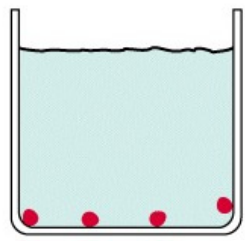
- Colour proportional to Antigen in patient sample.



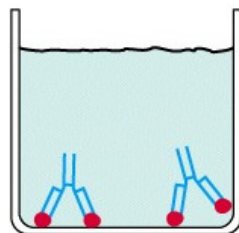


Indirect method

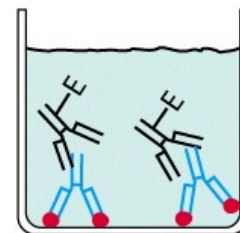
(Detection of Ab)



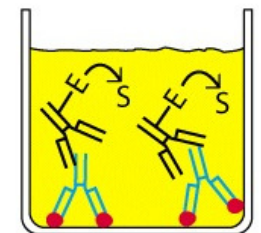
Antigen-coated well



Add specific antibody to be measured



Add enzyme-conjugated secondary antibody

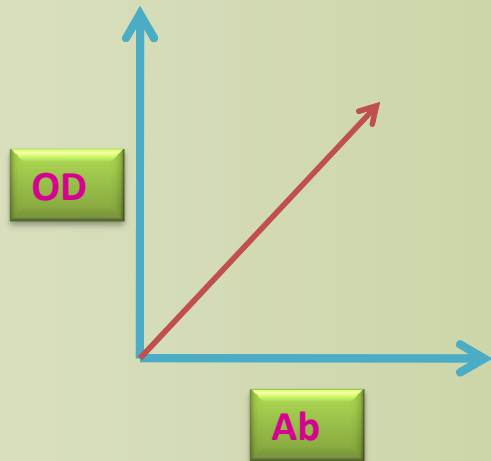


Add substrate (S) and measure color





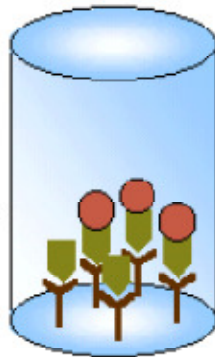
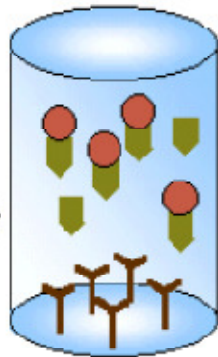
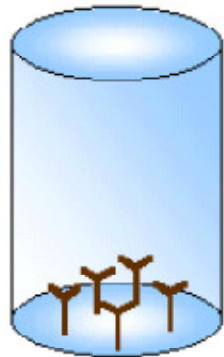
Colour proptrtional to Ab in patient sample.



Competitive method

Working Principle of the competitive ELISA

(ELISA) enzyme linked immunosorbent assay



Add 100 μ l unknown solution
& 100 μ l tracer solution

Remove excess
antigen



Reaction of the substrate



photometric detection



Antibody



Tracer (Antigen
+ Enzyme)



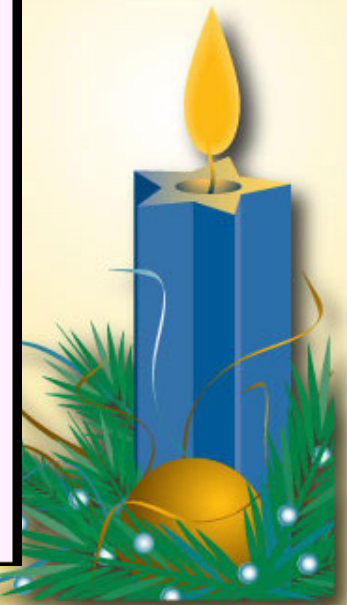
Antigen (Hormone)



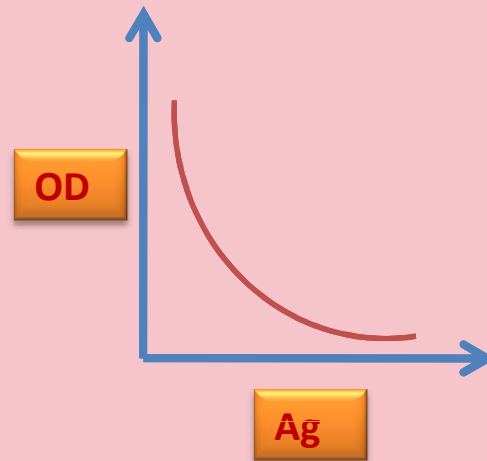
Substrate



modified Substrate
(coloured)



- Colour inversely related to Ag in patient serum.



Application Of ELISA

- ☀ Hormones in the serum like Thyroid hormones, Insuline....
- ☀ Tumer marker like AF1, PSA
- ☀ Infectious Disease like Bacterial toxin, Viruses, Hepatitis – B Surface antigen
- ☀ Assay of the Ab in serum infectious disease like Rubella Viruses, HIV etc...
- ☀ Assay of auto Ab or Anti DNA, anti-nuclear Abs etc...



- Advantage :**
- **No Radiation hazard**
 - **High sensitive**
 - **Obtain quick and accurate result**
 - **Minimal discomfort**
 - **Used in wide variety of test**
 - **It doesn't need costly instrumentation.**
 - **Antigens of very low or unknown concentration can be detected since capture antibody only grabs specific antigen.**



- Disadvantage :**
- **Monoclonal antibodies more difficult separate**
 - **Enzyme/substrate reaction is short term so Microwells must be read as soon as possible**
 - **Good Time management require**
 - **Monoclonal antibodies can cost**
 - **Require good skill**
 - **Require good quality of ELISA kit.**





RIA

Radioactive Hazard.

Used estimation of **very small** concentration.

Very **high cost** equipment

Cheap reagent.

Value measured in **curie & microcurie**

Require certificate & training from RIA centre.

ELISA

It is not Hazard.

Used estimation of **small** concentration.

Low cost equipment

Costly reagent.

Value is measured in **micro.**

No training nor certificate require.

