

# ELISA

## Enzyme Linked Immunosorbent Assay



# Learning Objectives:

- ◎ Basic ELISA principle.
- ◎ Brief History.
- ◎ Types of ELISA.
- ◎ ELISA Applications.
- ◎ Practical part (ELISA virtual lab).

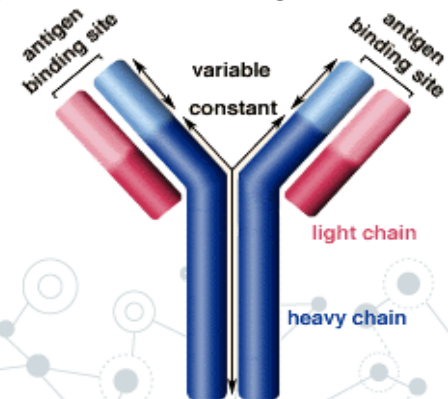


# Key Terminology:

## Antibodies:

specialized soluble proteins produced by B cells and plasma cells that interact with antigen; also called immunoglobulin (Ig).

- ✓ Each B-cell makes its own distinct antibody in response to a specific antigen.
- ✓ Each antibody is designed to bind to a specific surface binding site or **epitope** on the antigen.
- ✓ There are millions of different types of antibodies circulating in an individual's bloodstream and they are based on exposure to antigens in his/her environment.



# Key Terminology:

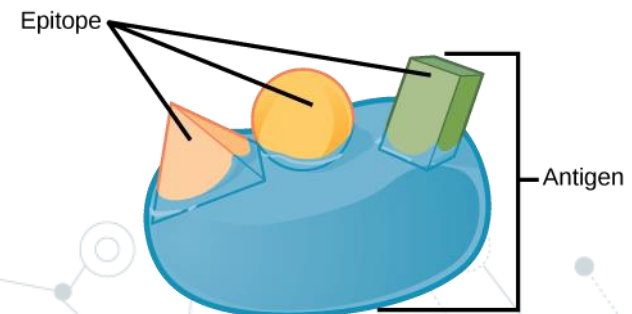
## Antigens:

substances that when introduced into the body stimulates the production of an antibody.

**Antigens = “non-self” molecules and cells**

such as:

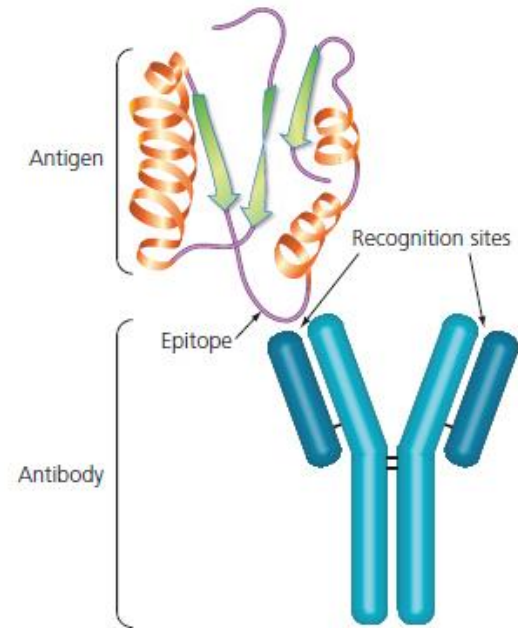
- foreign proteins
- viruses
- environmental pollutants
- bacteria and parasites (Protista, Fungi, Plantae, and Animalia cells).
- foreign transplanted tissue
- cancerous cells



# Key Terminology:

## Immunoassay:

A laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample.



The background of the slide is a light gray network diagram. It consists of numerous small circles, some solid and some hollow, connected by thin lines. The circles are arranged in a complex, interconnected pattern, resembling a molecular structure or a data network. The overall aesthetic is clean and technical.

**What is ELISA?**

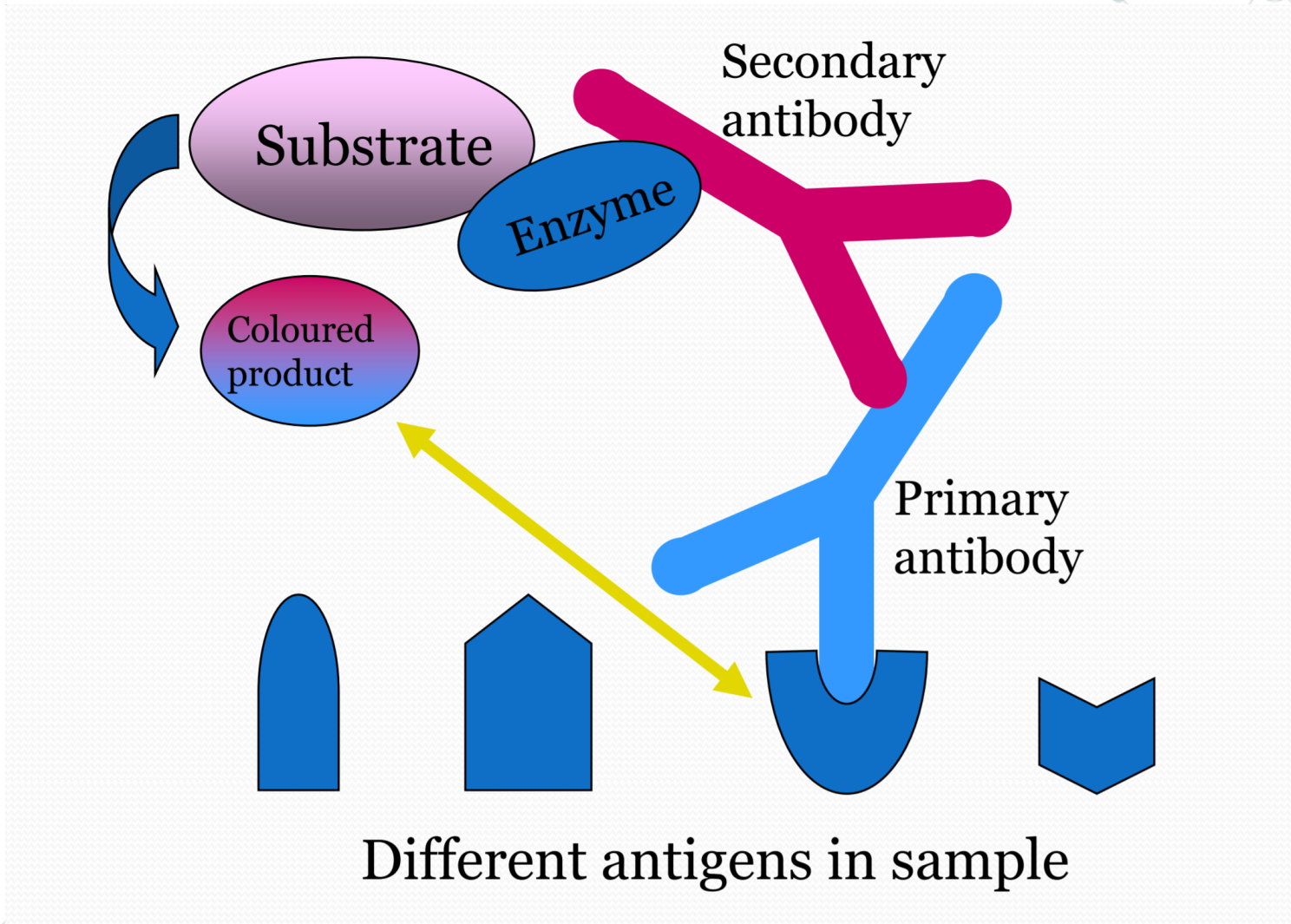


“

# ELISA

## Enzyme Linked Immunosorbent Assay

is a sensitive immunochemical technique which used to detect and quantify a specific protein (**antigen** or **antibody**) in a given sample. Other names, such as **Enzyme Immunoassay (EIA)**, are also used to describe the same technology. The reaction is measurable in both **qualitative** and **quantitative** terms.





# ELISA

Enzyme Linked Immunosorbent Assay

Primary antibody is recognised by second antibody which has enzyme attached  
**(enzyme linked).**

Antigen is recognised by specific antibody  
**(immuno).**

Antigen of interest is absorbed on to plastic surface  
**(sorbent).**



“

## History

In **1960s** the only option for conducting an immunoassay was radioimmunoassay (RIA), a technique using radioactively labeled antigens or antibodies. Because radioactivity poses a potential health threat, a safer alternative was required.



“

## History

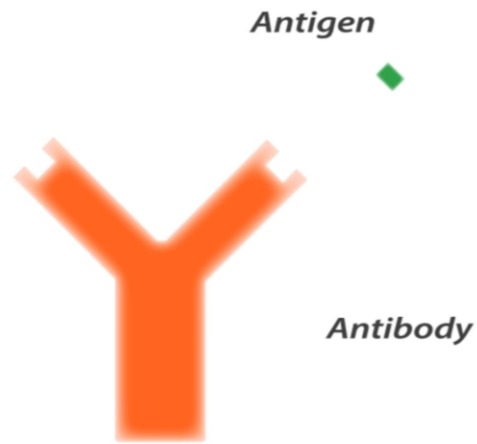
In **1971s** Two scientific research groups independently and simultaneously developed this idea (The principle of immunoassay with an enzyme rather than radioactivity as the reporter label). The ELISA technique was conceptualized and developed by **Perlmann and Engvall** in **Sweden**, and the EIA technique by **Schuurs and van Weemen** in **Netherlandsand** and secured patents on their findings.



**Fig. 1.** Preis Biochemische Analytik, Munich, April 1976. From left to right, Dr. Eva Engvall (Sweden), Dr. Anton Schuurs (The Netherlands), Dr. Peter Perlmann (Sweden), Dr. Bauke van Weemen (The Netherlands)

# Basic principle





The identification happens by **antigen $\leftrightarrow$ antibody interaction**

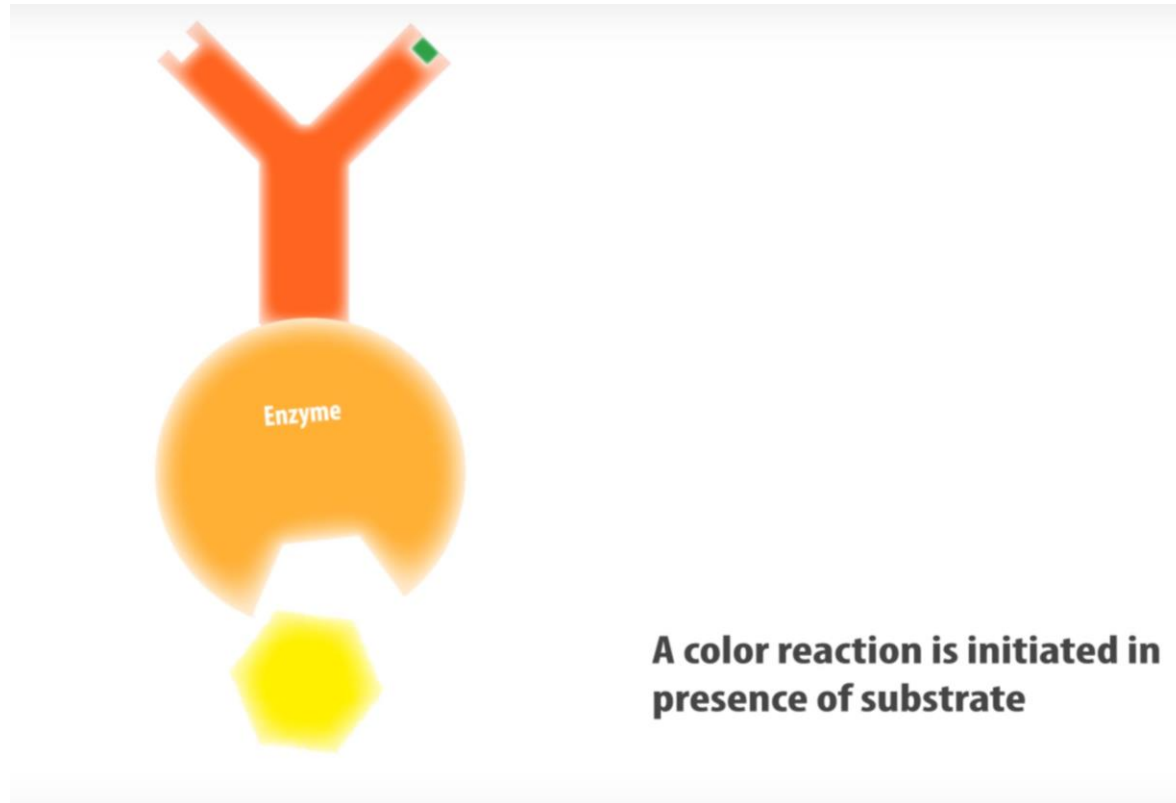


**The antibody is linked with an enzyme**



**A color reaction is initiated in presence of substrate**





enzyme converts colourless substrate (**chromogen**) to a coloured product, indicating the presence of Ag : Ab binding.

# ELISA Types:

There are five types\* of ELISA methods which include:

- ⦿ Indirect ELISA
- ⦿ Sandwich ELISA
- ⦿ Direct ELISA
- ⦿ Competitive ELISA
- ⦿ Multiplex ELISA

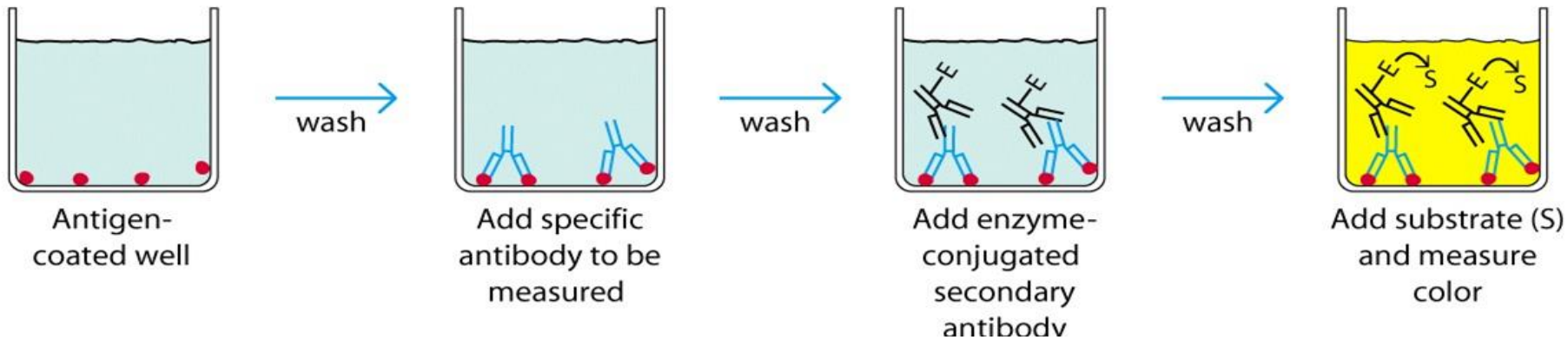
\* The indirect (to detect antibodies) and the sandwich (to detect antigens) ELISA methods are the two most common types used.



# Indirect ELISA

to detect Ab, (example: HIV, HCV)

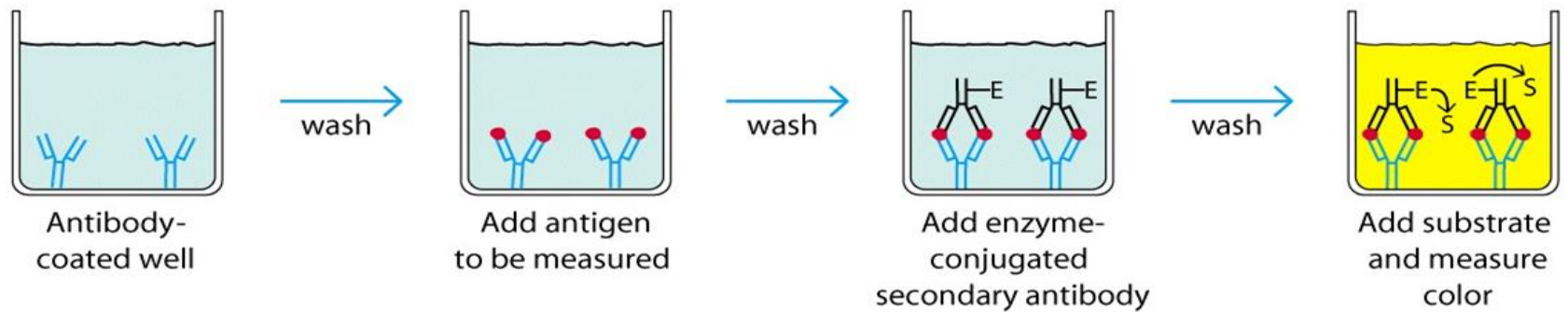
(a) Indirect ELISA



# Sandwich ELISA


to detect Ag, (example: Tumour Markers, Hormones )

(b) Sandwich ELISA

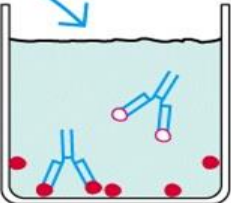


# Competitive ELISA

(c) Competitive ELISA

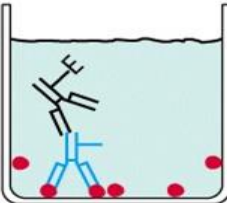


Incubate antibody with antigen to be measured



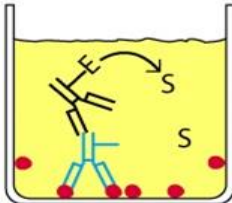
Add Ag-Ab mixture to antigen-coated well

wash



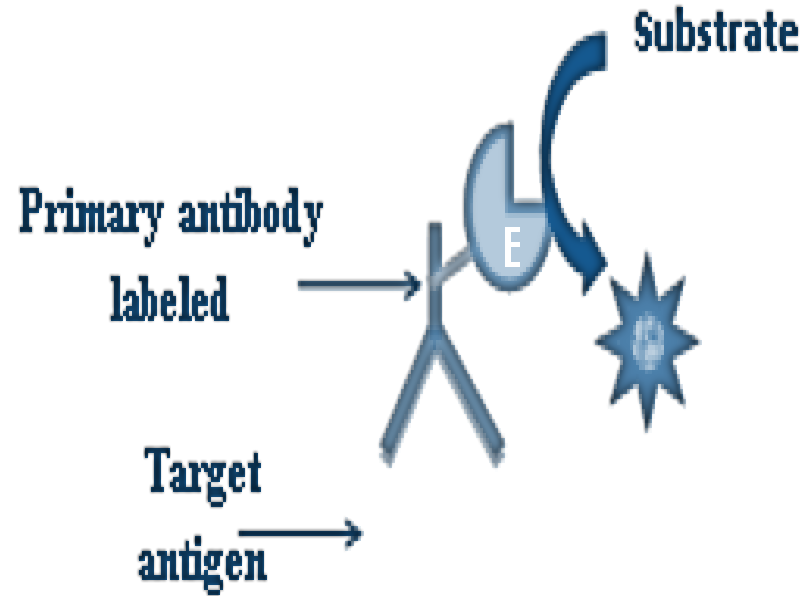
Add enzyme-conjugated secondary antibody

wash



Add substrate and measure color

# Direct ELISA



Direct ELISA



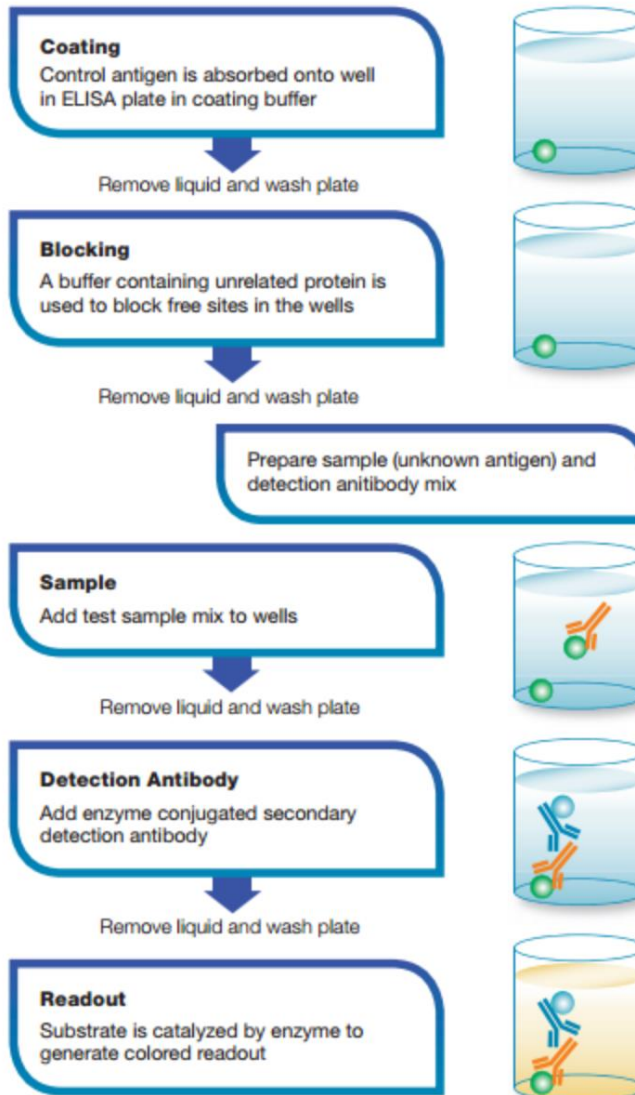
# Multiplex ELISA

Up to 50 protein in one assay!!!

uses magnetic beads that have specific antibodies on their surface. Each magnetic bead is color-coded with a unique spectral signature



# Basic Steps Of ELISA





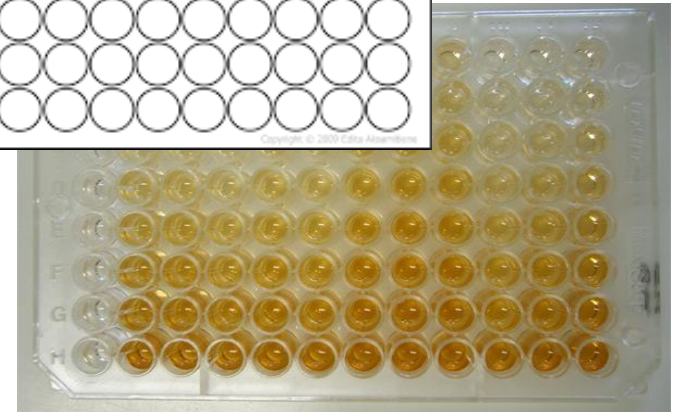
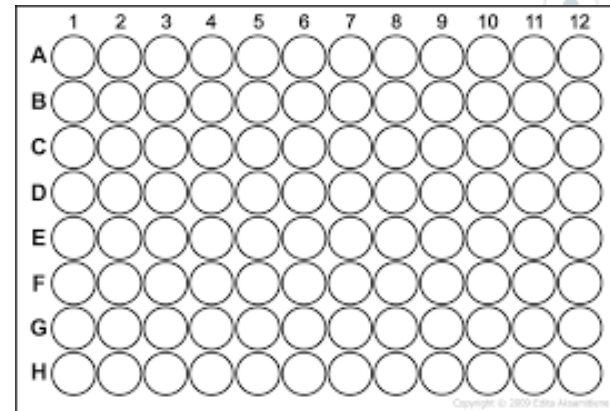
# Requirements for ELISA test:

- Purified antigen → (if you want to detect or quantify antibody).
- Purified antibody → (if you want to detect or quantify antigen).
- Standard solutions (**positive** and **negative** controls).
- Sample to be tested (**blood**, **urine**, **CSF**, **Sputum....**).
- Micro-titer plates.
- Wash fluid (**buffer**).
- Enzyme-labeled antibody and enzyme substrate.
- ELISA reader.





**Fig. 3.** ELISA/EIA Test Kits



**Fig. 3. ELISA Reader Spectrophotometer, A microplate reader with a 96-well plate in the sample drawer**

# Advantages vs. Disadvantages

highly specific and sensitive

Reagents are relatively cheap & have a long shelf life

No radiation hazards occur during labelling or disposal of waste.

can be used to a variety of infections.

Easy to perform and quick procedures

Measurement of enzyme activity can be more complex than measurement of activity of some type of radioisotopes.

Kits are commercially available, but not cheap

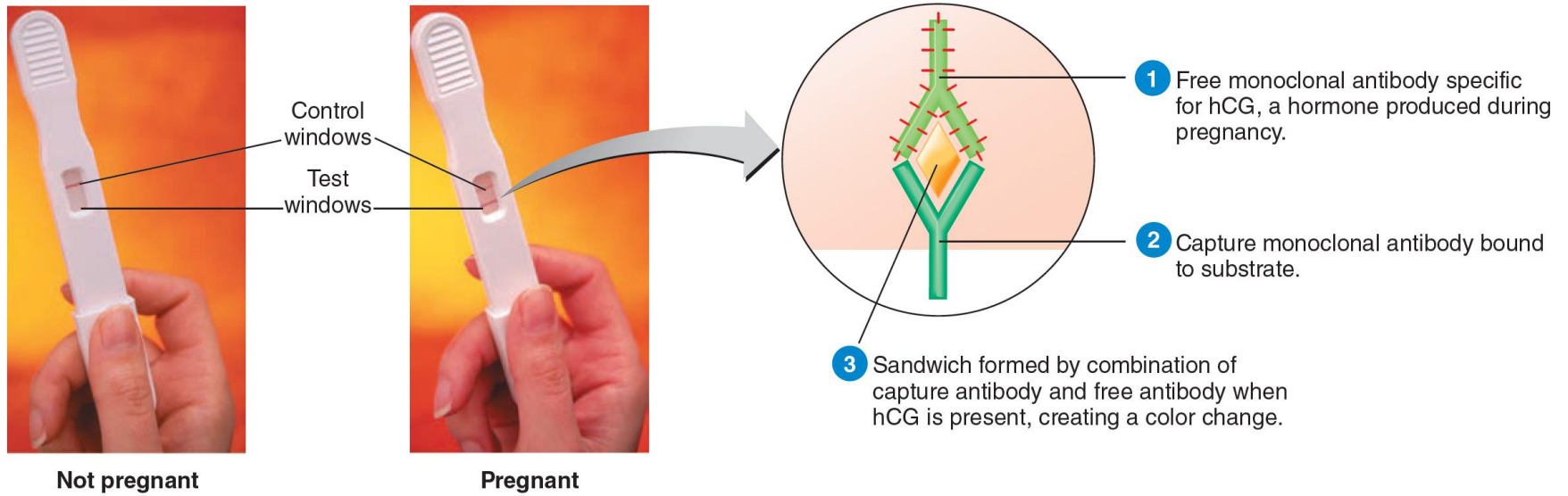
Very specific to a particular antigen. Won't recognize any other antigen

False positives/negatives possible, especially with mutated/altered antigen

Antibody must be available.

# ELISA Applications

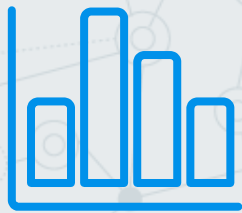




**Figure 18.13 The use of monoclonal antibodies in a home pregnancy test.** Home pregnancy tests detect a hormone called human chorionic gonadotropin (hCG) that is excreted only in the urine of a pregnant woman.

## Some ELISA Applications:

- Antibody Concentration Determination.
- Virus test (HIV, West Nile Virus, Hepatitis B and C).
- Home Pregnancy Test
- Food industry (detecting potential food allergens such as milk, walnuts, almonds and eggs)
- Parasitic infection (Toxoplasmosis).
- toxicology as a rapid presumptive screen for certain classes of drugs.
- *Helicobacter pylori*
- autoimmune diseases



6.845%

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**The Virtual Immunology Lab**  
Howard Hughes Medical Institute

**Summary**

This virtual lab teaches the procedures of performing an ELISA test to determine whether a particular antibody is present in a patient's blood sample.

[Start Virtual Lab](#)

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Components of the immune system called antibodies are found in the liquid portion of blood and help protect the body from harm. Antibodies can also be used outside the body in a laboratory-based assay to help diagnose disease caused by malfunctions of the immune system or by infections.

The following browsers are supported and recommended for this virtual lab. Please be sure that JavaScript and cookies are enabled and your pop-up blocker is disabled.

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**Worksheet (PDF)**  
71 KB

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## Practical part (ELISA virtual lab)

<https://www.hhmi.org/biointeractive/immunology-virtual-lab>



# Thanks!

## Any questions?

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*Email:* [maljumaah1@ksu.edu.sa](mailto:maljumaah1@ksu.edu.sa)

