ELISA

Enzyme Linked Immunosorbent Assay

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Learning Objectives:

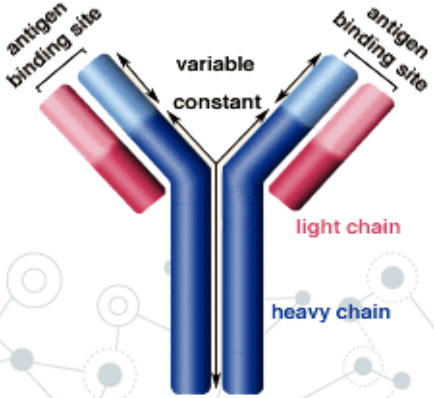
- Basic ELISA principle.
- Brief History.
- Types of ELISA.
- ELISA Applications.
- Practical part (ELISA virtual lab).

Key Terminology:

Antibodies:

specialized soluble proteins produced by B cells and plasma cells that interacts 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:

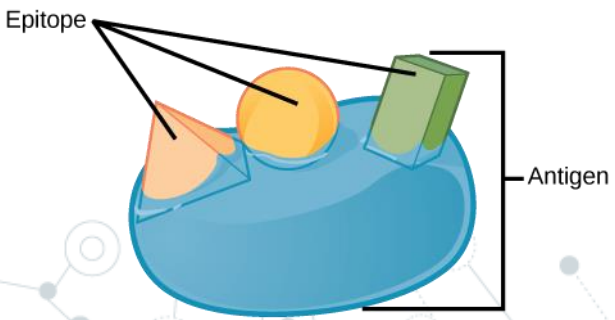
Antigens:

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

Antigens = “non-self” molecules and cells

such as:

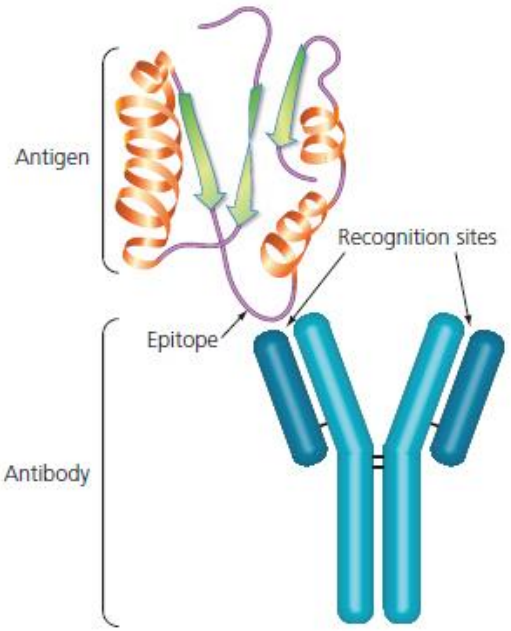
- foreign proteins
- viruses
- environmental pollutants
- bacteria and parasites (Protista, Fungi, Plantae, and Animalia cells).
- foreign transplanted tissue
- cancerous cells



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.

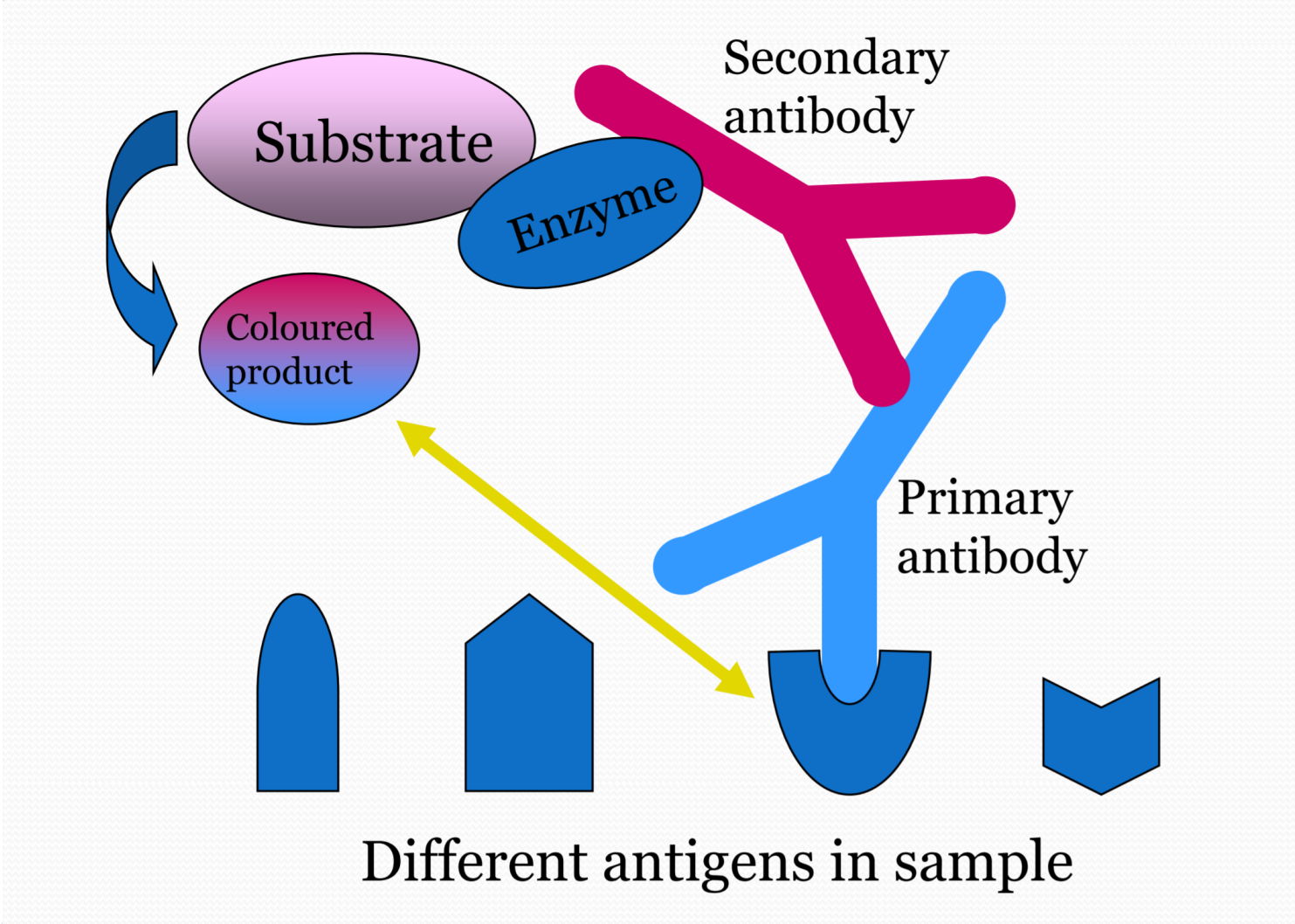


What is ELISA?

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.


Secondary antibody
Substrate
Enzyme
Coloured product
Primary antibody
Different antigens in sample

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

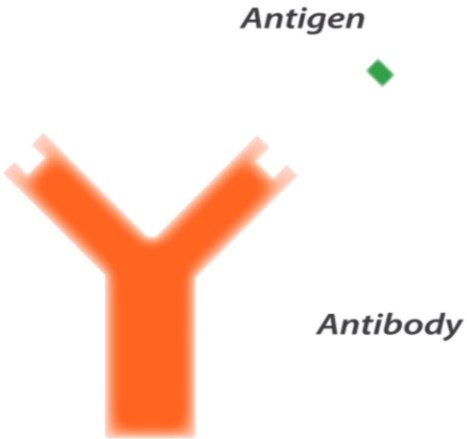
In **1960s** the only option for conducting an immunoassay was radioimmunoassay (RIA), a technique using radioactively labeled antigens or antibodies. Because radioactivity poses a potential health threat, a safer alternative was required.

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)

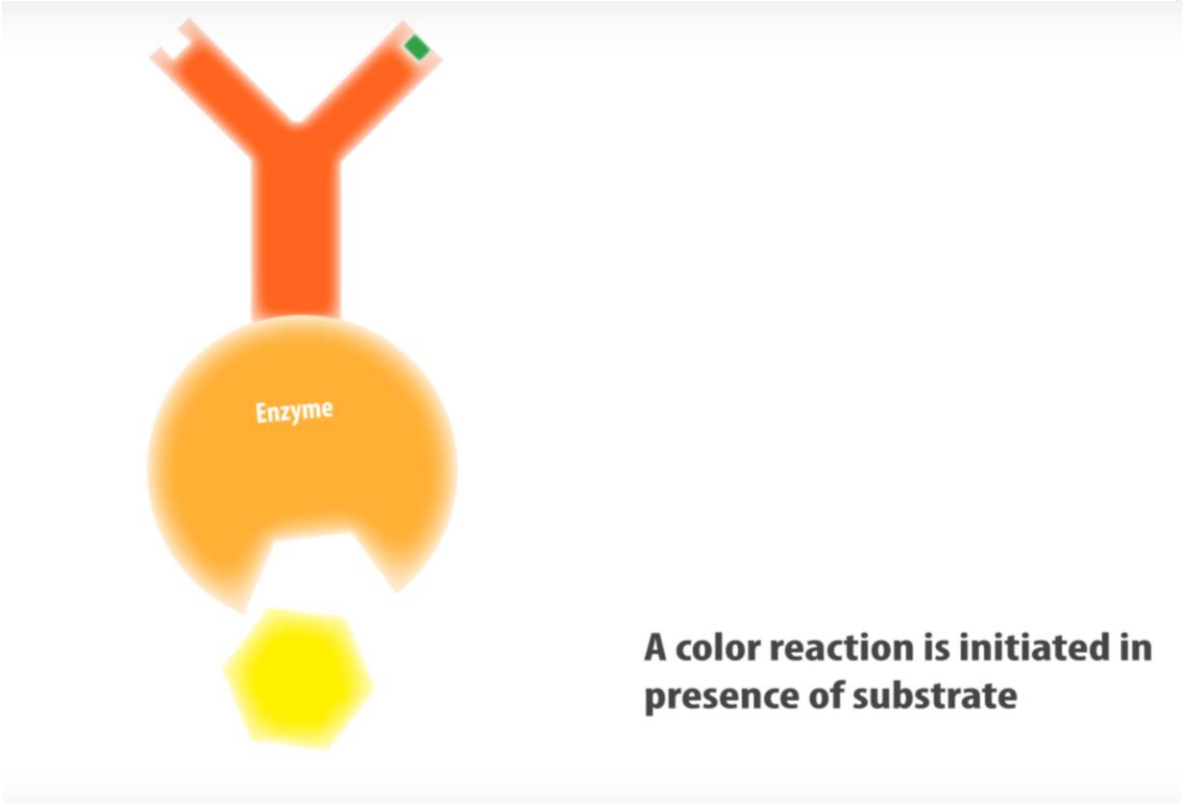
Basic principle



The identification happens by antigen<-->antibody interaction

The antibody is linked with an enzyme

A color reaction is initiated in presence of substrate

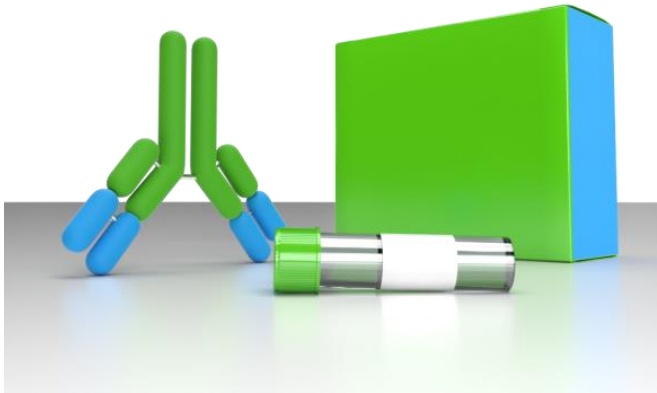


enzyme converts colourless substrate **(chromogen)** to a coloured product, indicating the presence of Ag : Ab binding.

ELISA Types:

There are five types* of ELISA methods which include:

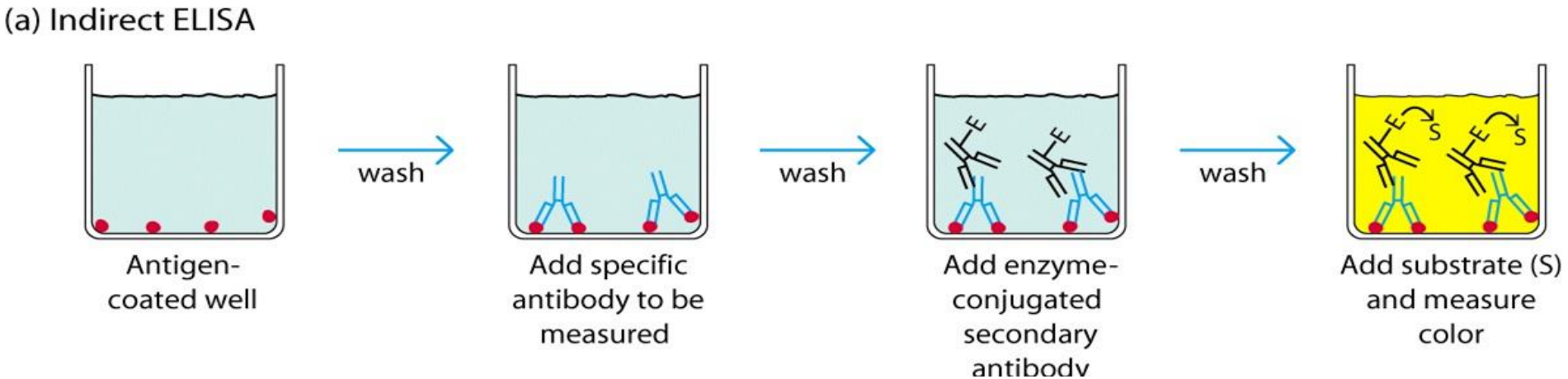
- Indirect ELISA
- Sandwich ELISA
- Direct ELISA
- Competitive ELISA
- Multiplex ELISA



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

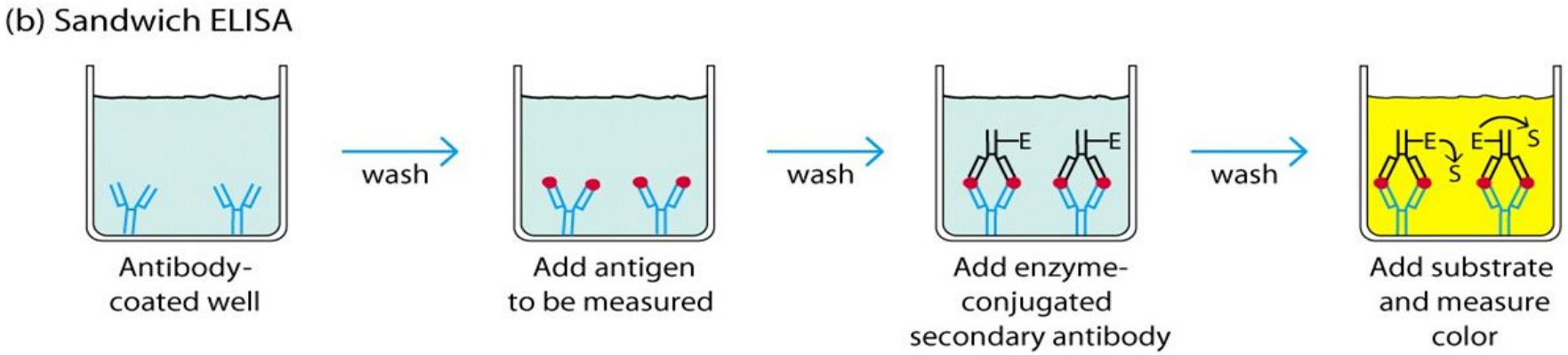
Indirect ELISA

to detect Ab, (example: HIV, HCV)

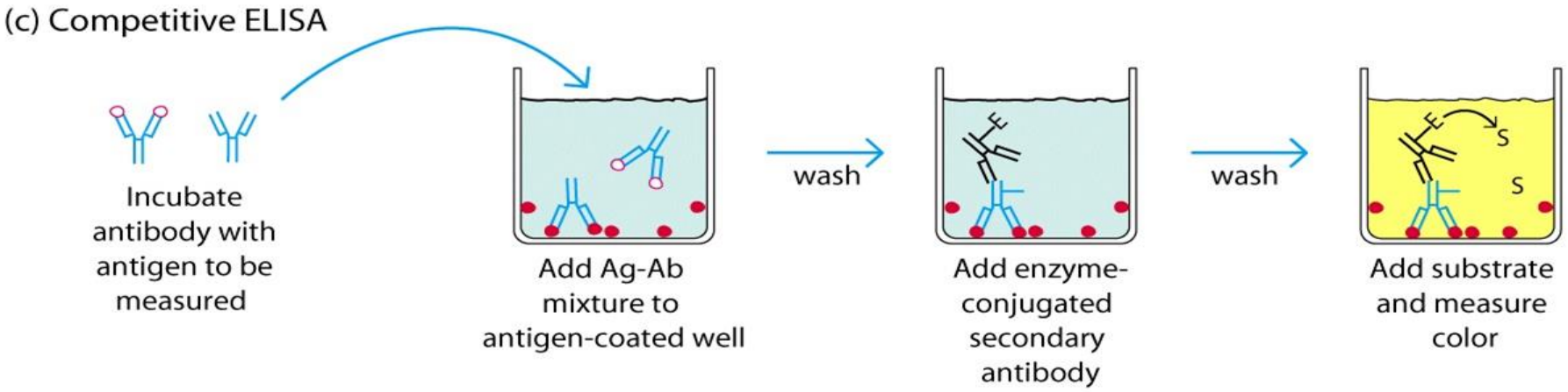


Sandwich ELISA

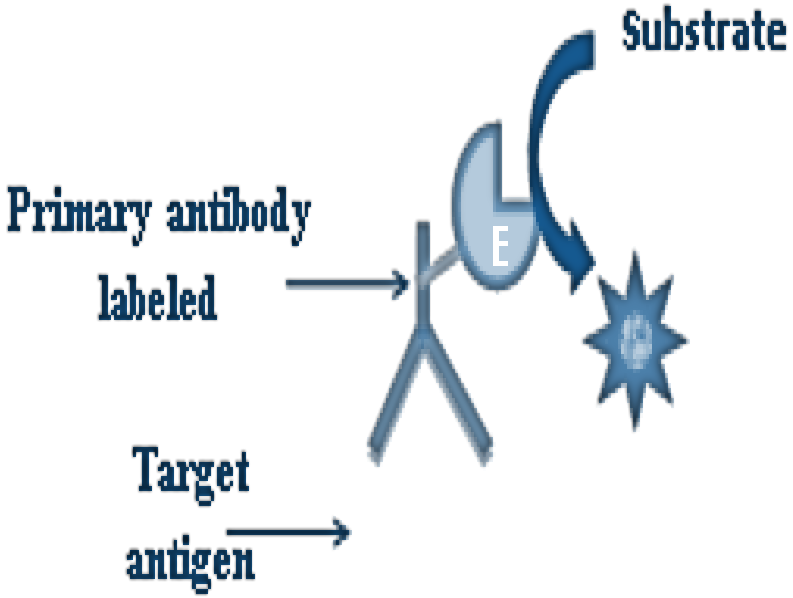
to detect Ag, (example:Tumour Markers, Hormones)



Competitive ELISA



Direct ELISA

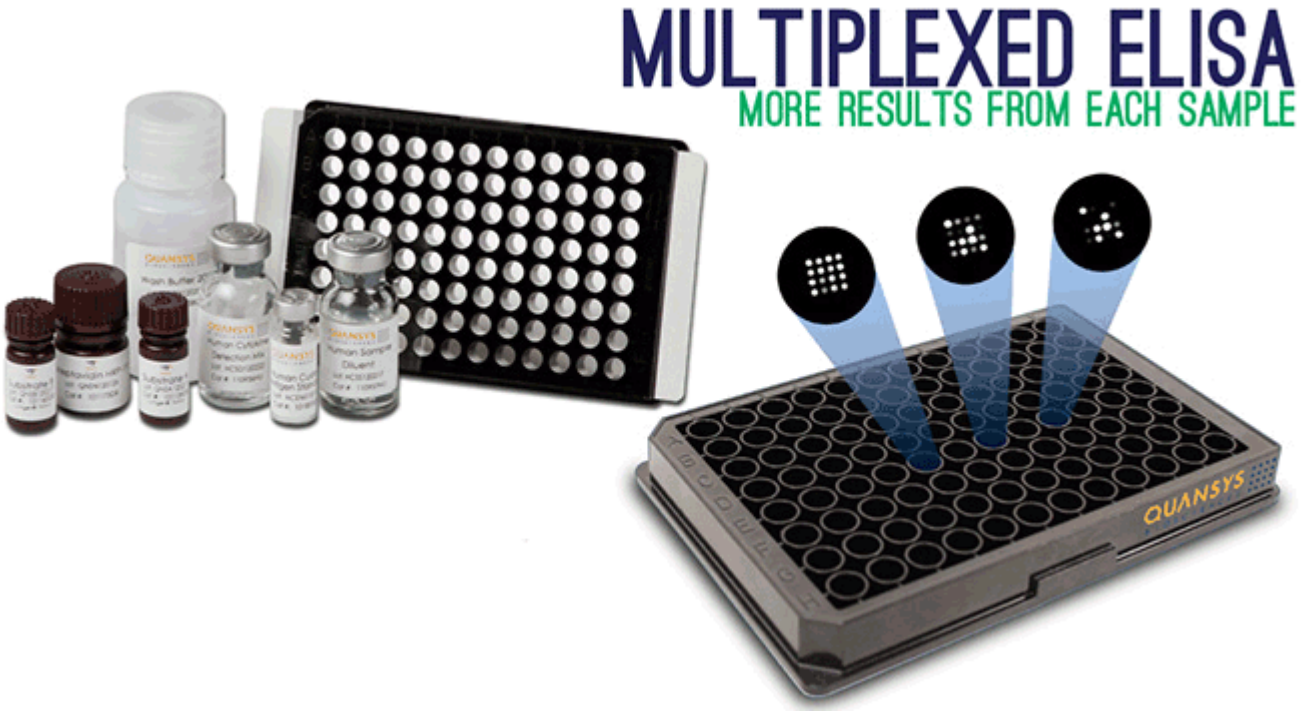


Direct ELISA

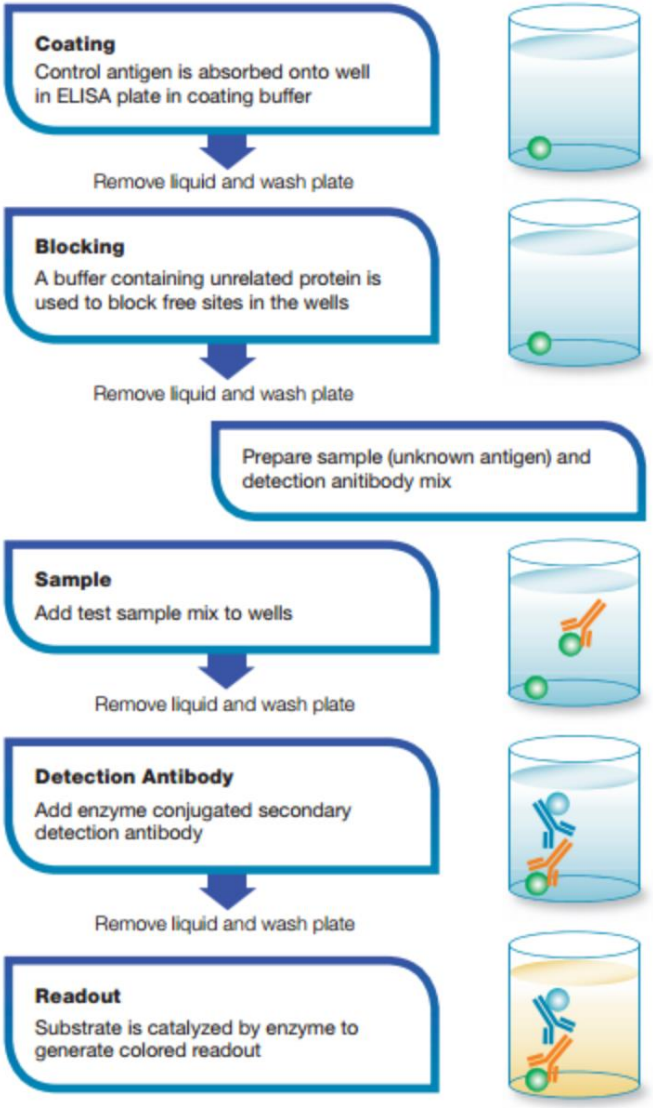
Multiplex ELISA

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



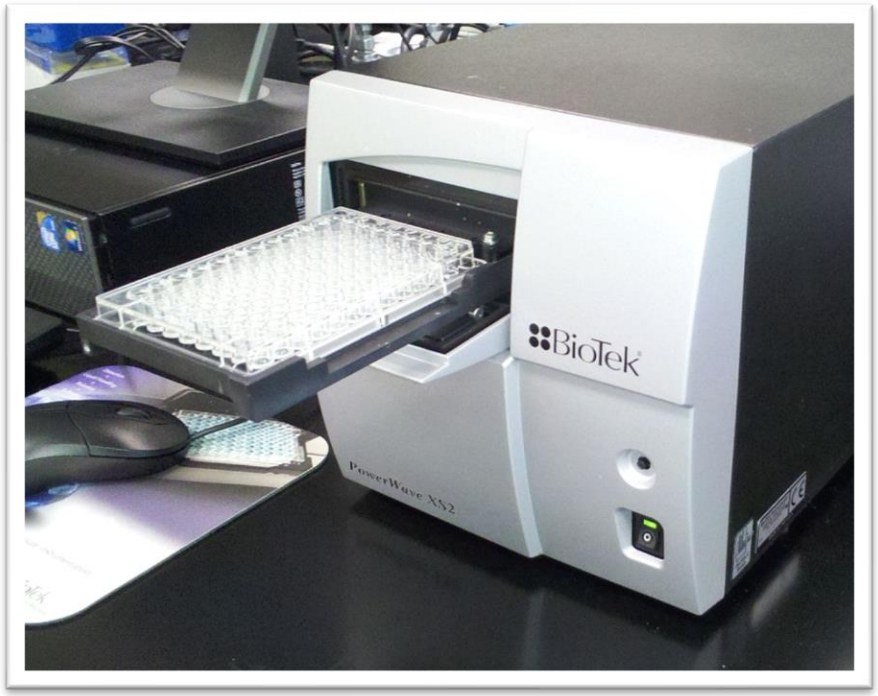
Basic Steps Of ELISA



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



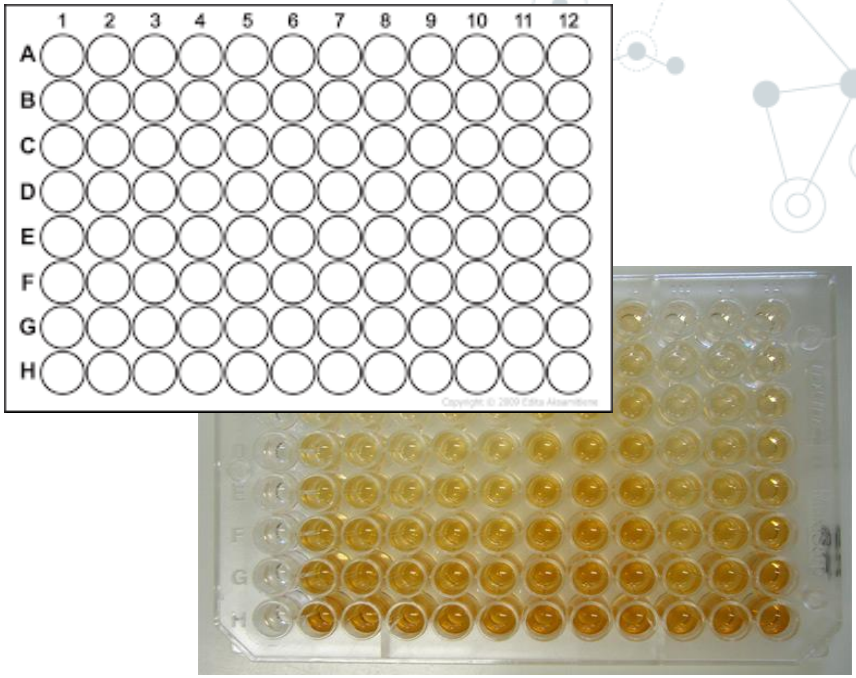


Fig. 3. ELISA Reader Spectrophotometer, A microplate reader with a 96-well plate in the sample drawer

Advantages vs. Disadvantages

highly specific and sensitive	Measurement of enzyme activity can be more complex than measurement of activity of some type of radioisotopes.
Reagents are relatively cheap & have a long shelf life	Kits are commercially available, but not cheap
No radiation hazards occur during labelling or disposal of waste.	Very specific to a particular antigen. Won't recognize any other antigen
can be used to a variety of infections.	False positives/negatives possible, especially with mutated/altered antigen
Easy to perform and quick procedures	Antibody must be available.

ELISA Applications

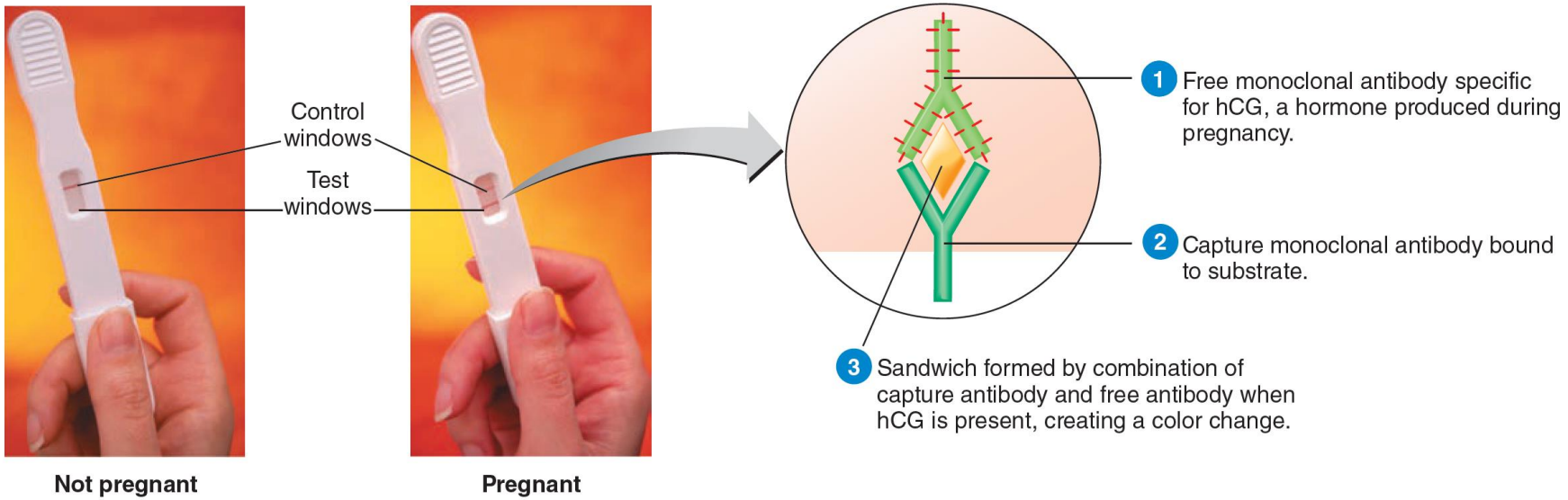


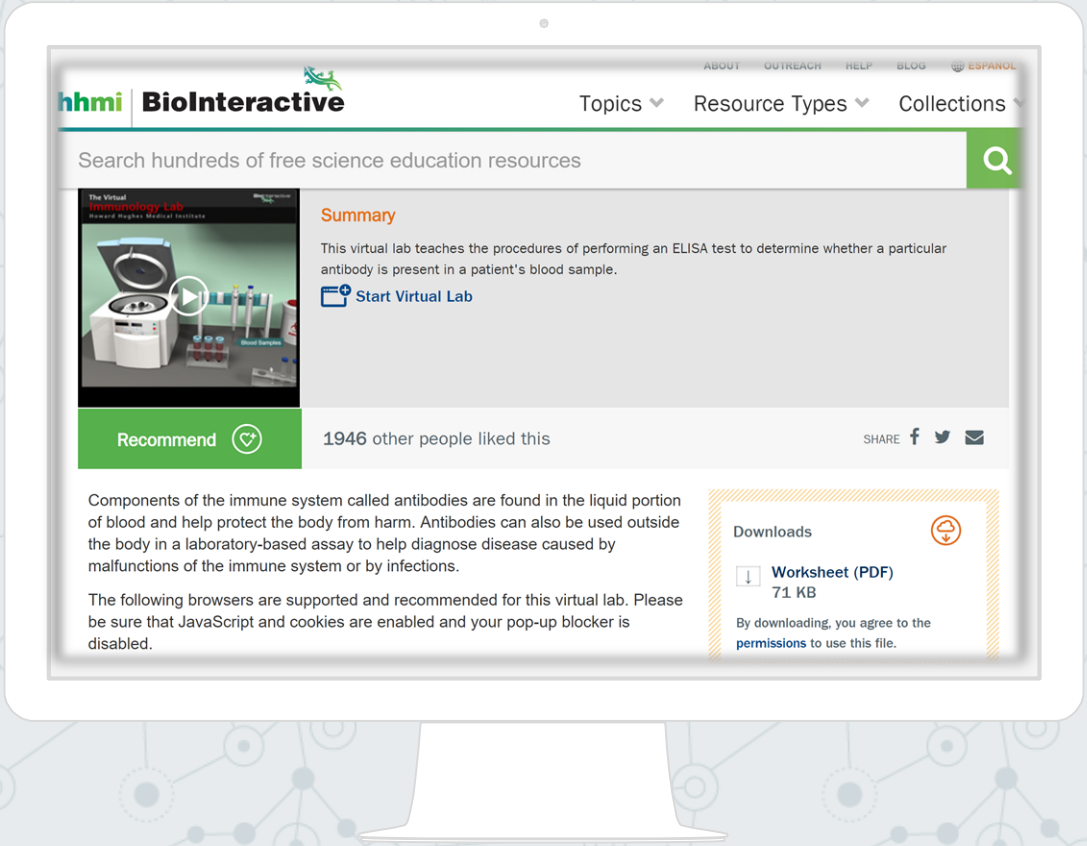
Figure 18.13 The use of monoclonal antibodies in a home pregnancy test. Home pregnancy tests detect a hormone called human chorionic gonadotropin (hCG) that is excreted only in the urine of a pregnant woman.

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)
- Pparasitic infection (Toxoplasmosis).
- toxicology as a rapid presumptive screen for certain classes of drugs.
- *Helicobacter pylori*
- autoimmune diseases

6.845%

The number of people in the world that is educated to the level equivalent to the Bachelor's Degree.



Practical part (ELISA virtual lab)

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

Thanks!

Any questions?

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