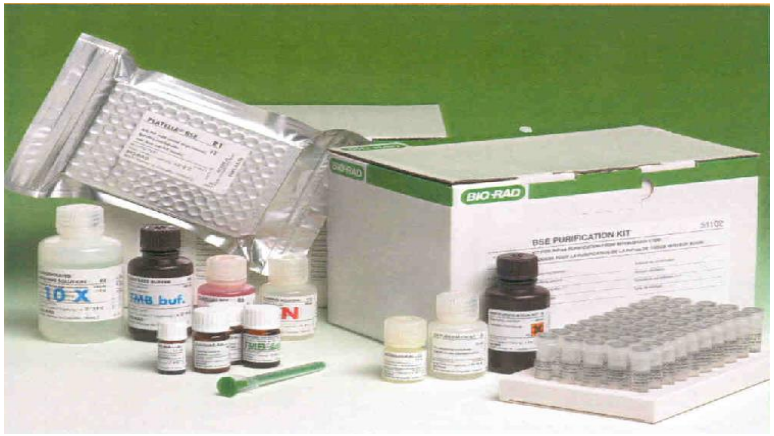
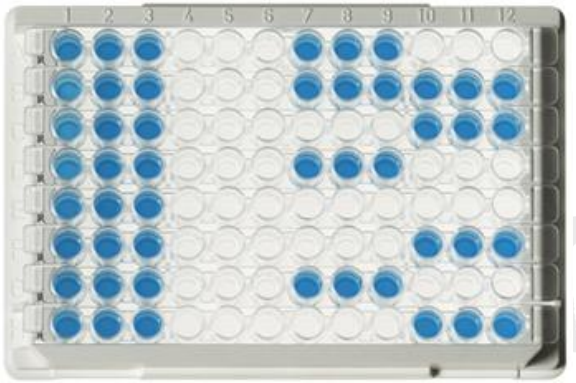
ELISA

Enzyme-Linked Immune Sorbent Assay





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The purpose of the workshop

- History of Elisa
- **Enzyme-Linked Immune Sorbent Assay**
- **What Are Antigens?**
- What Are Antibodies and How Are They Produced?
- Hum oral (Antibody-Mediated) Immune System
- Types of ELISA Methods
- Immunohistochemical Staining
- Elisa animation

History of Antibody-Antigen Interactions 1

In 1890, Emil von Behring from Germany noticed an “antitoxin” formed in the blood of animals infected with diphtheria bacillus. When antitoxin serum was transferred to animal given a lethal dose of toxin, the animal survived.

They proposed the humoral theory of immunity based on their toxin-antitoxin theory.

In 1901, Emil Von Behring was awarded the first Nobel Prize in Physiology or Medicine “for his work on serum therapy, especially its application against diphtheria”.

History of Antibody-Antigen Interactions - 2

In 1891, Paul Erlich was the first to use the term **“antikorper”,** the German word for antibody, in an article he wrote.

In 1897, he proposed the idea that the “side chain” receptors on the surface of cells could bind to specific toxins in a “lock-and-key” interaction.

Paul Erlich shared the Nobel Prize in Physiology or Medicine in 1908 with Ilya Mechnikov, a Russian scientist, “in recognition for their work on immunity”.

History of Antibody-Antigen Interactions - 3

In 1940, Linus Pauling at the California Institute of Technology confirmed the lock-and-key theory proposed by Erlich and was awarded the Nobel Prize in Chemistry in 1954 “for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances” including antibodies and the nature of serological reactions.

In 1948, Astrid Fagreaus at the Karolinska Institutet in Stockholm, Sweden presented evidence that Blymphocytes in the form of plasma cells formed the antibodies circulating in the bloodstream.

History of Antibody-Antigen Interactions - 4

By the 1960s, Gerald Edelman at Rockefeller University in New York and Rodney Porter at the University of Oxford, England worked out the structure and complete amino acid sequence of the antibody, Iggy.

In 1972, Gerald Edelman and Rodney Porter were shared the Nobel Prize in Physiology or Medicine “for their discoveries concerning the chemical structure of antibodies”. .

RadioImmunoAssay (RIA)

Based on their understanding of the specificity and affinity of the antigen-antibody interaction, Solomon Berson and Rosalyn Yalow at the Veterans Administration Hospital in New York developed a method called Radioimmuno Assay (RIA) in 1960.
RIA was used to measure the amount of endogenous plasma insulin by tagging the insulin with a radioactive label.

Originally, they used iodine-131, half-life=8.1 days, a beta and gamma emitter. Later, for safety reasons, iodine-125, halflife=60.14 days, weak gamma emitter, was used instead.
Radioimmuno Assays opened the door for others to develop similar methods, like ELISA tests in 1971.

History of ELISA/EIA

The development of the radioimmunoassay opened the door for others to develop similar methods, like ELISA, to test for the presence of proteins but without the use of radioactive substances.

In 1971, Peter Permann and Eva Engvall in Stockholm published the first paper on Enzyme-Linked ImmunoSorbent Assay (ELISA) showing they could quantify the amount of IgG in rabbit serum using alkaline phosphatase (an enzyme) as the reporter label. The same year, Anton Schuurs and Bauke Van Weemen in the Netherlands published a paper showing that with an Enzyme ImmunoAssay (EIA), they could quantify the amount of human chorionic gonadotropin in urine with horseradish peroxidase (an enzyme) coupled with glutaraldehyde as the reporter label.

The assays were highly sensitive and compared favorably with Radioimmuno Assays.

ELISA

Enzyme-Linked Immune Sorbent Assay.

- The ELISA assay is a widely used biochemical assay to detect presence of and quantity of compounds, such as hormones and antibodies and bacteria or viruses in a the sample
- The ELISA assay uses the coupling of antigens and antibodies and relies on the specificity and affinity of antibodies for antigens.
- Specificity is the ability to discriminate among diverse proteins. Affinity is the ability to tightly bind to molecules.
- 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.

What Are Antigens?

Antigens are any foreign substance in the body. Antigens include “not-self” molecules and cells, such as:

a. foreign proteins

b. viruses

c. environmental pollutants and other foreign substances like asbestos, tattoo ink, and cigarette smoke

d. bacteria and parasites (Protista, Fungi, Plantae, and Animal cells)

e. foreign transplanted tissue

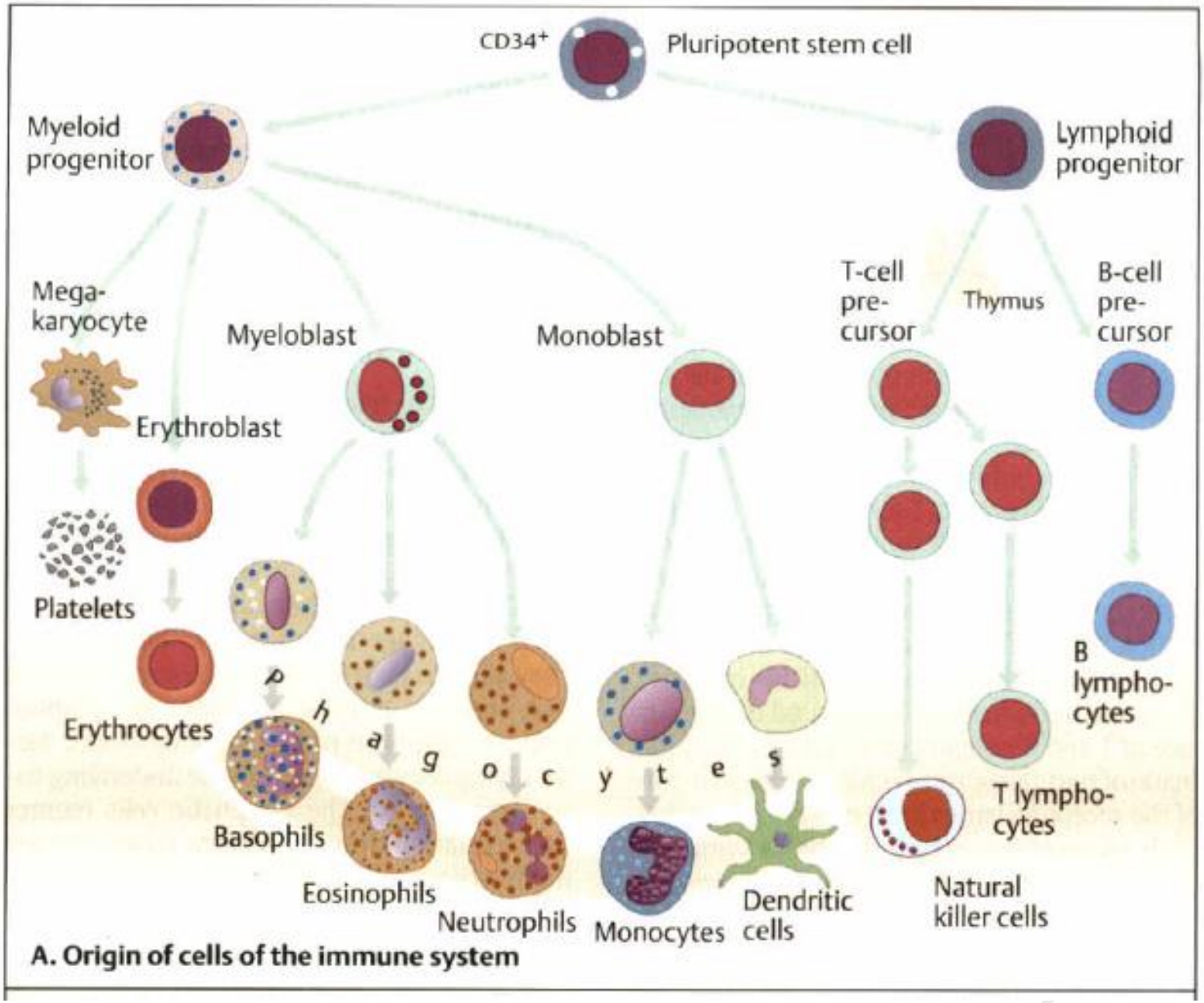
f. cancerous cells

What Are Antibodies and How Are They Produced?

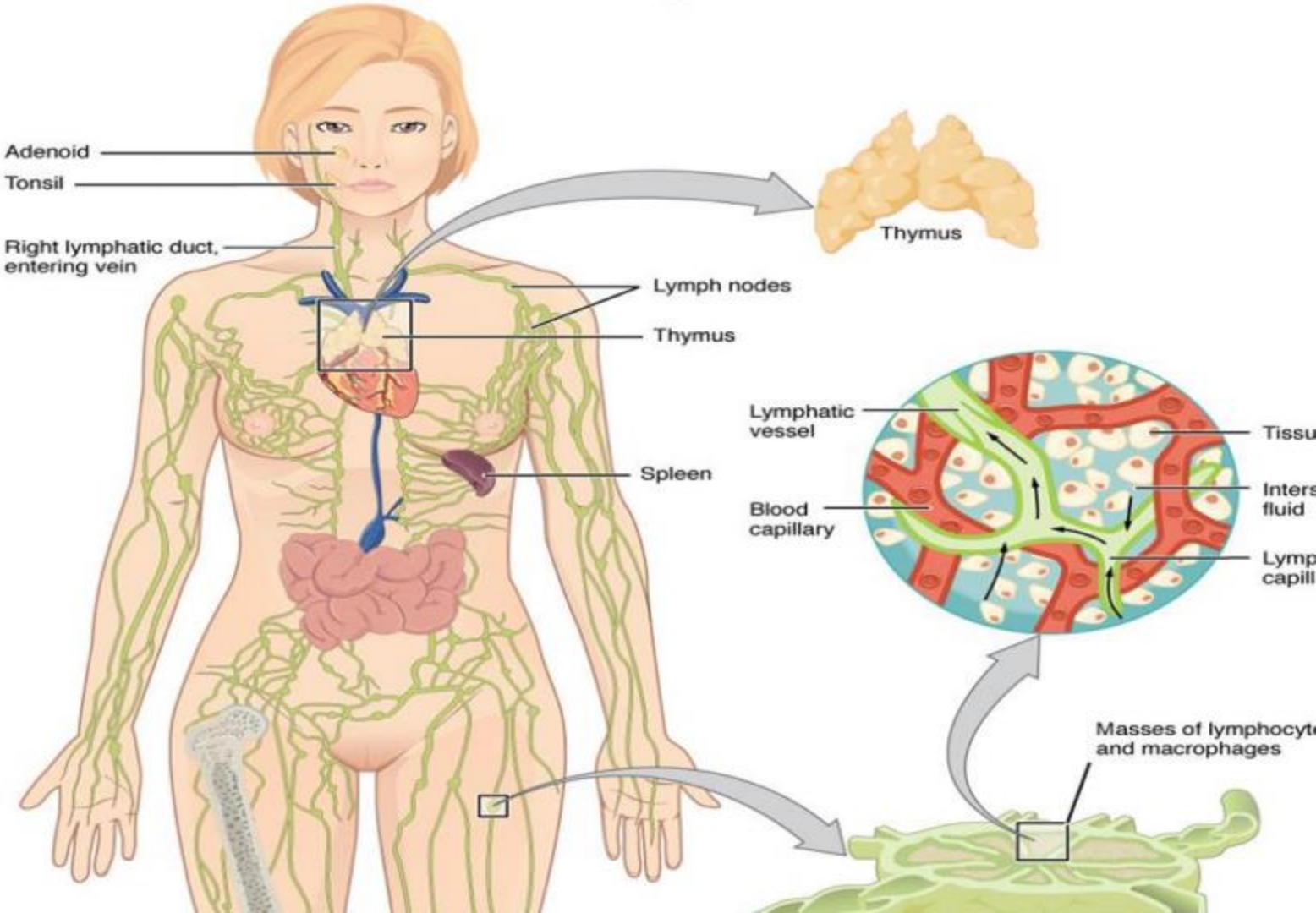
Antibodies are large glycoprotein molecules produced by B-lymphocytes during the humoral immune response to antigens introduced into the body.

Lymphocytes include B-lymphocytes (B-cells) and Tlymphocytes (T-cells) which are white blood cells form from the hematopoietic (blood) stem cells in the bone marrow.

The immune system is made of two parts – humoral (antibody-mediated) and cellular (cell-mediated).


CD34+
Pluripotent stem cell
Myeloid progenitor
Lymphoid progenitor
Mega-karyocyte
Erythroblast
Myeloblast
Monoblast
T-cell pre-cursor
Thymus
B-cell pre-cursor
Platelets
Erythrocytes
Basophils
Eosinophils
Neutrophils
Monocytes
Dendritic cells
B lympho-cytes
T lympho-cytes
Natural killer cells
A. Origin of cells of the immune system

B Lymphocytes Mature in the Bone Marrow an
Lymphocytes Mature in the Thymus Gland

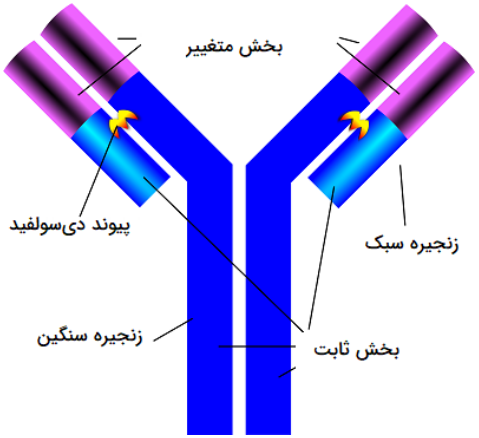


Hum oral (Antibody-Mediated) Immune System

B-lymphocytes produce large glycoproteins called antibodies in response to antigens (any foreign substance) and then mark those antigens-antibody complex to be destroyed by the T-lymphocytes.

Each B-cell makes its own distinct antibody in response to a specific antigen which comes in contact with it. Each antibody is designed to bind to a specific surface binding site or epitope on the antigen.

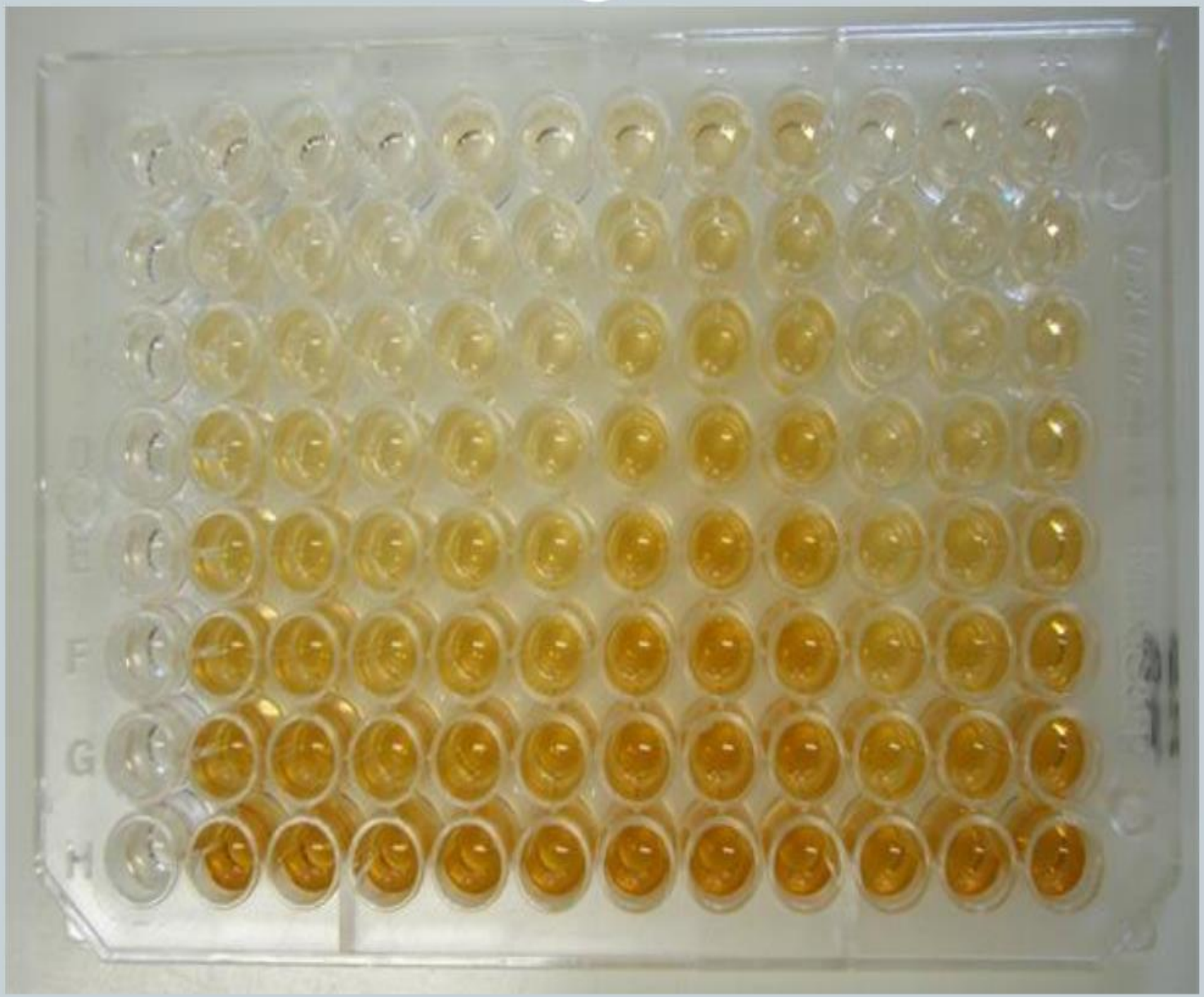
There are millions of different types of antibodies circulating in an individual's bloodstream and they are based on exposure to antigens in his/her environment.



Over 80% of human glycoprotein antibodies are in the immunoglobulin class IgG. They are shaped like a Y and are found in the blood, lymph, and intestine.

IgG molecules have a molecular weight of 150,000 Daltons and are made of 2 long (heavy) chains coded from chromosome 14, and 2 short (light) chains coded from either chromosome 2 or 22, and then all connected by disulphide bonds.

Most of the molecule is composed of a constant region that doesn't change from one IgG molecule to another. However, the ends of the Y are variable, which accounts for each IgG molecule binding only to a specific antigen.


F
G
H

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

Types of ELISA Methods

The ELISA method has been used to detect hepatitis B, rabies, and HIV through antibodies in the blood serum, or to measure the amount of various other proteins in the blood serum, such as hormones, toxins, and allergens.

There are five types of ELISA methods which include:

Indirect ELISA

Sandwich ELISA

Direct ELISA

Competitive ELISA

Multiplex ELISA

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

The Indirect ELISA Method – Part 1

a) Binding Known Antigen - The indirect ELISA method begins with a sample of known antigen being bound to the wells of a microtiter plate.

b) Blocking - The other unoccupied sites in each well are then bound by a concentrated solution of non-interacting protein, like casein or bovine serum albumin, to block or prevent other proteins in the test sample from adhering.

c) Washing – Rinse to remove any unbound antigen and non-interacting protein.

The Indirect ELISA Method – Part 1

d) Adding Test Sample Primary Antibody - The test sample of serum containing the primary antibodies is added to each well. Antibodies could be for example, HIV, rabies, or hepatitis B antibodies.

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

The Indirect ELISA Method – Part 2

f) Adding Enzyme-linked Secondary Antibody - 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.

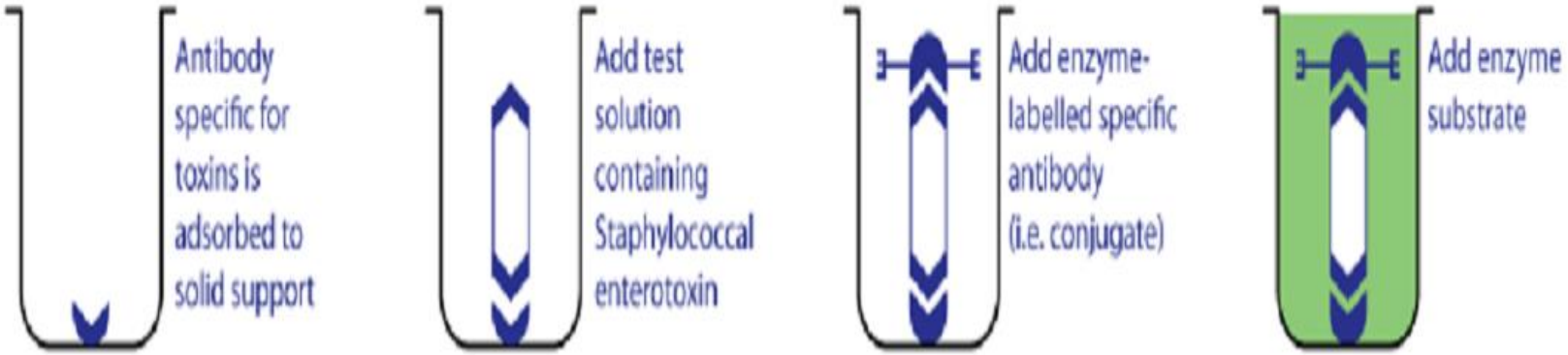
g) Washing – Rinse to remove any secondary antibodies that did not bind to the primary antibody.

The Indirect ELISA Method – Part 2

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 turns green, OPD turns orange, and TMB turns blue. In the presence of alkaline phosphatase, pNPP turns yellow.

i) Reading Results - By using a spectrophotometer, spectrofluorometer, or electrochemical device, the results can be read and recorded. The amount of color produced is proportional to the amount of primary antibody bound to the antigen proteins on the bottom of the wells

Sandwich ELISA



As the substrate is added, the enzyme converts it into a colored product. The rate of color formation is proportional to the amount of antigen present.

Immunohistochemical Staining

While the ELISA tests are routinely used to test antigen and antibody presence in patient blood serum, the direct ELISA and indirect ELISA methods have also been applied in immunohistochemistry.

The tissue being studied would be embedded in paraffin and thinly sliced with a microtome onto a glass microscope slide.

Immunohistochemical Staining

In order to fluorescently tag a particular cell component, the paraffin would be removed, the antigens of the tissues retrieved, and a blocking non-interacting protein would be added to bind all unoccupied sites on the slide.

Then the slide would be washed to remove any unbound noninteracting protein.

From there, either the direct or indirect method would be applied.

Indirect Method-Immunohistochemical Staining

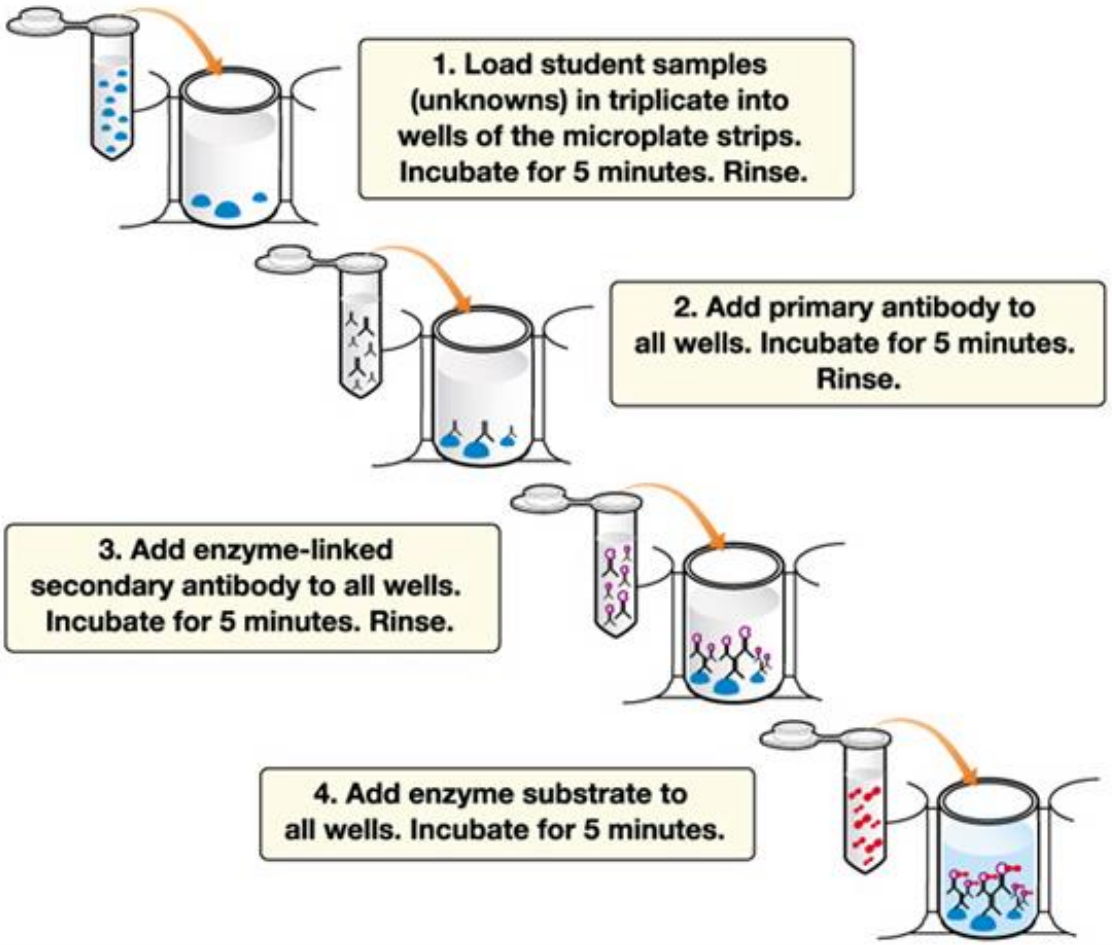
After deparaffinization, antigen retrieval, blocking, and washing, an antigen-specific primary antibody is added and then washed to remove any unbound primary antibody.

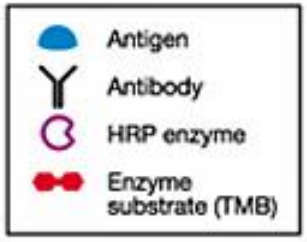
Then an enzyme-linked secondary antibody is added and then washed to remove unbound secondary antibodies. As substrate is added, the interaction between the substrate and enzyme occurs and the cell component becomes visible.

While the direct immunohistochemical method is much quicker, the indirect immunohistochemical method is thought to be more sensitive.

ELISA ANIMATION

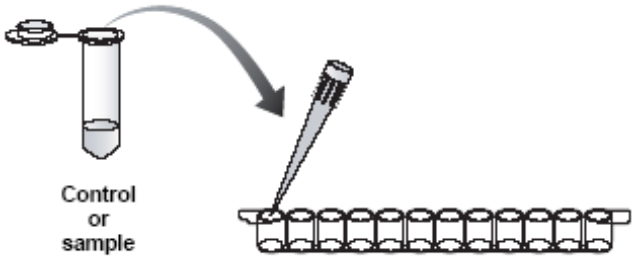
ELISA Procedures Overview



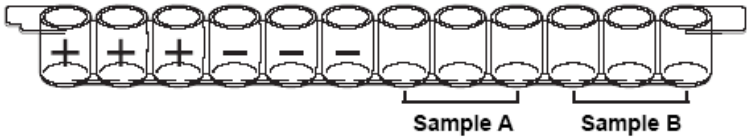


Step One

Label and add controls

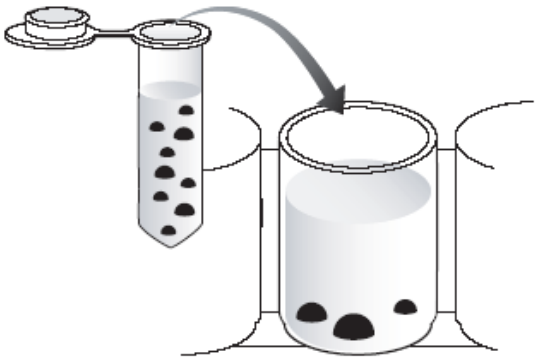


- **Obtain a test-sample**
- **Label the 12-well strip:**
  - **First 3 wells: positive controls "+"**
  - **Next 3 wells: negative controls "-"**
  - **Remaining wells to identify test-samples**



- **Add 50 ul of positive control to 1st 3 wells**
- **Add 50 ul of negative control to 2nd 3 wells**
- **Add 50ul of the student samples to the appropriately labeled wells**
- **Wait 5 minutes for the antigen to bind**

Microplate Strips

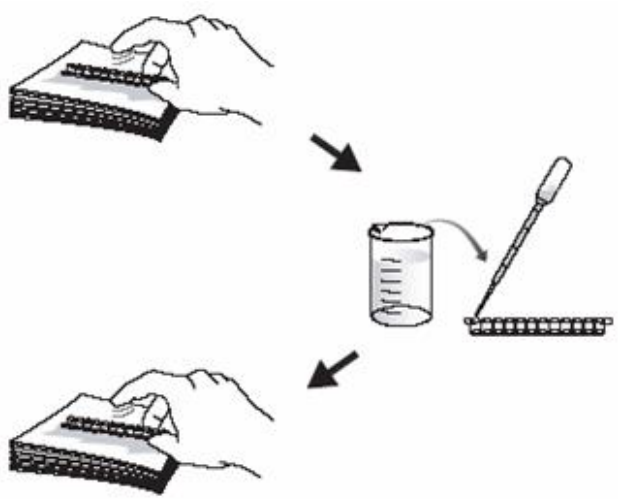


- **Microplate strips are made of polystyrene**
- **Hydrophobic side chains in amino acids bind to the polystyrene wells**

- **No coating is needed**

Step Two

WASH

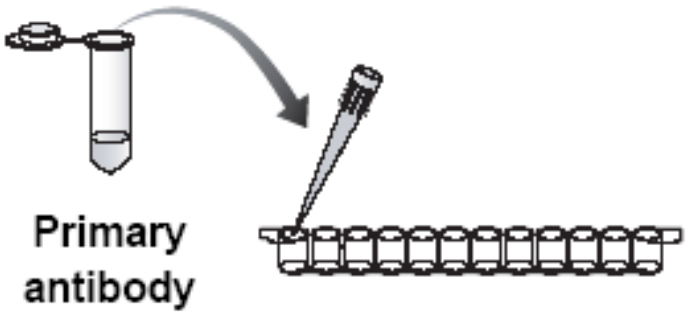


- Remove samples from wells by firmly tapping them on a paper towel
- Discard the top paper towel
- Using a disposable transfer pipette wash wells with wash buffer
- Remove wash buffer by firmly tapping the wells on a paper towel
- Discard the top paper towel
- Repeat wash step

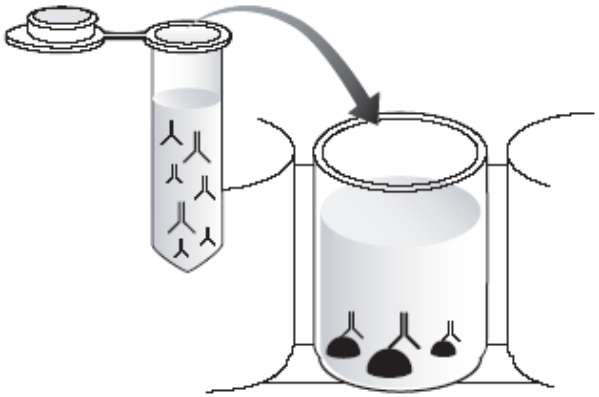
Step Three

Add (PA)
Primary Antibody

- **Add 50 ul of the primary antibody (PA) to all 12 wells**
- **Samples are left in wells for 5 minutes**
- **After 5 minutes WASH 2X**



Wash Buffer

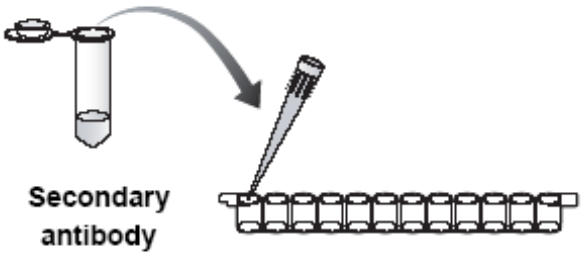


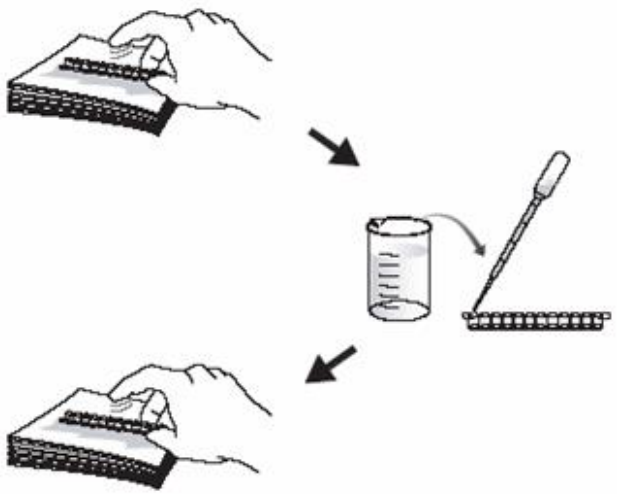
- **Wash buffer contains phosphate buffer saline (PBS) to keep antibodies in a stable environment that helps keep their structure**
- **Also contains Tween 20: a nonionic detergent removes non-specifically bound proteins and coats wells that acts as a blocking agent to reduce background**
- **Antibody will only bind to the antigen**

Step Four

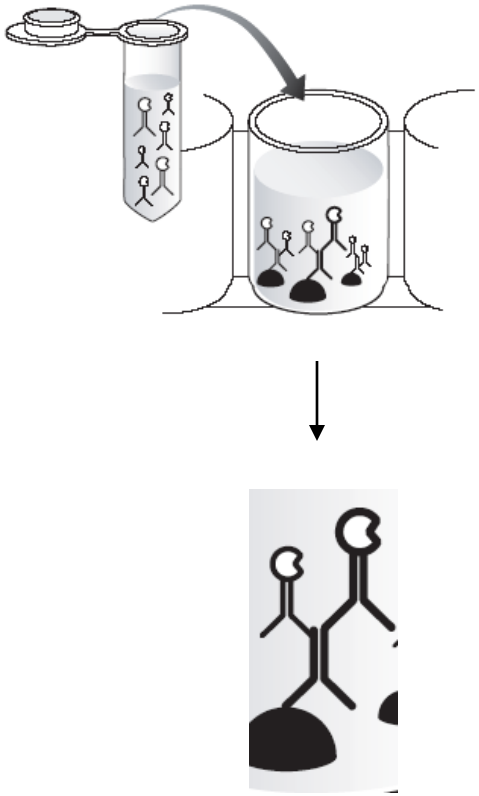
Wash antibody and add enzyme-linked secondary antibody (SA)

- Wash the primary antibody from polystyrene wells as before
- WASH 2X
- Add 50ul of the enzyme-linked secondary antibody to each well
- Wait 5 minutes

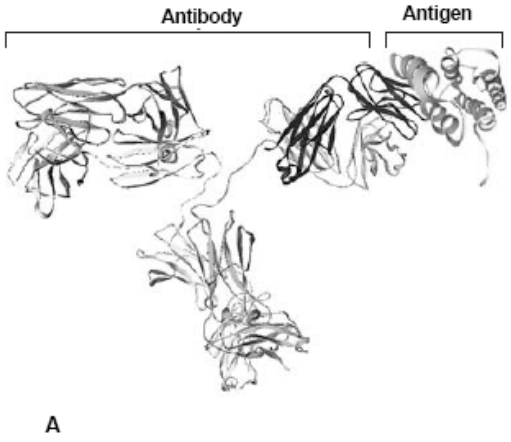


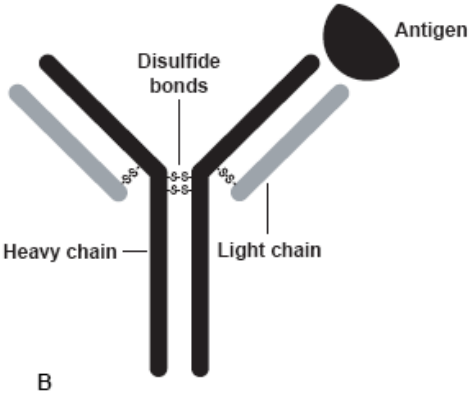


Antibody Specificity



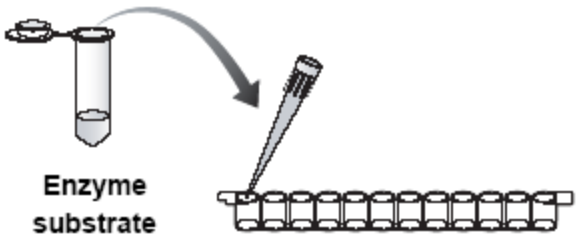
- **Secondary antibody (enzyme-linked antibody) will only bind to the primary antibody (serum antibody)**
- **Secondary antibody specifically recognizes the constant region of the primary antibody**
- **In which wells do you predict this is happening?**



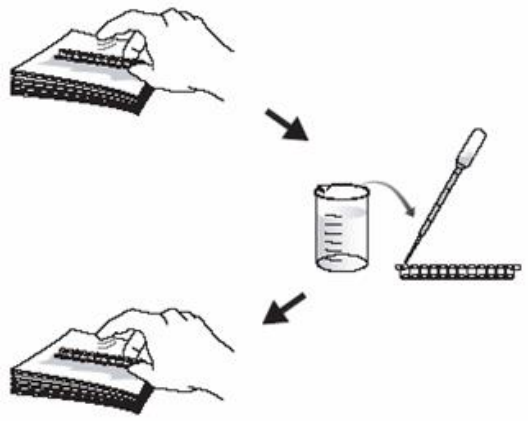


Step Five

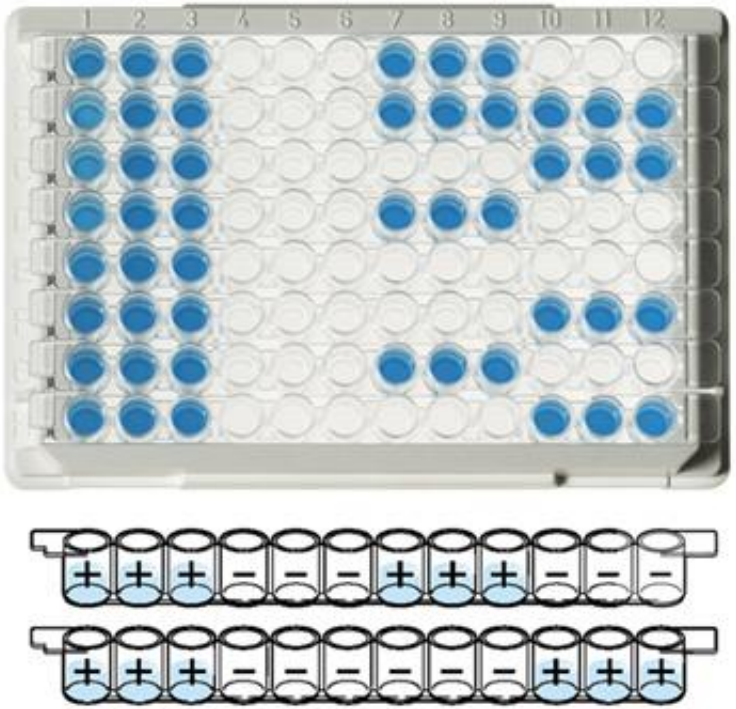
Add enzyme substrate (SUB)



- **Wash the enzyme-linked secondary antibody from polystyrene wells as before**
- **Using a disposable transfer pipette wash wells with wash buffer**
- **WASH 3X**
- **Add 50ul of the enzyme substrate to each well**
- **Wait 5 minutes**
- **positive samples will begin to turn blue**



ELISA Kit
Results




برای موفقیت،
«نمیدانم»
را به
«هنوز نمیدانم»
تغییر دهید.