

# Serology-Techniques

Serology refers to using antigen-antibody reactions in the laboratory for diagnostic purposes. Its name comes from the fact that serum, the liquid portion of the blood where antibodies are found is used in testing. Serologic testing may be used in the clinical laboratory in two distinct ways:

**a. To identify unknown antigens (such as microorganisms).** This is called direct serologic testing. **Direct serologic testing uses a preparation known antibodies, called antiserum,** to identify an unknown antigen such as a microorganism.

# Serology-Techniques

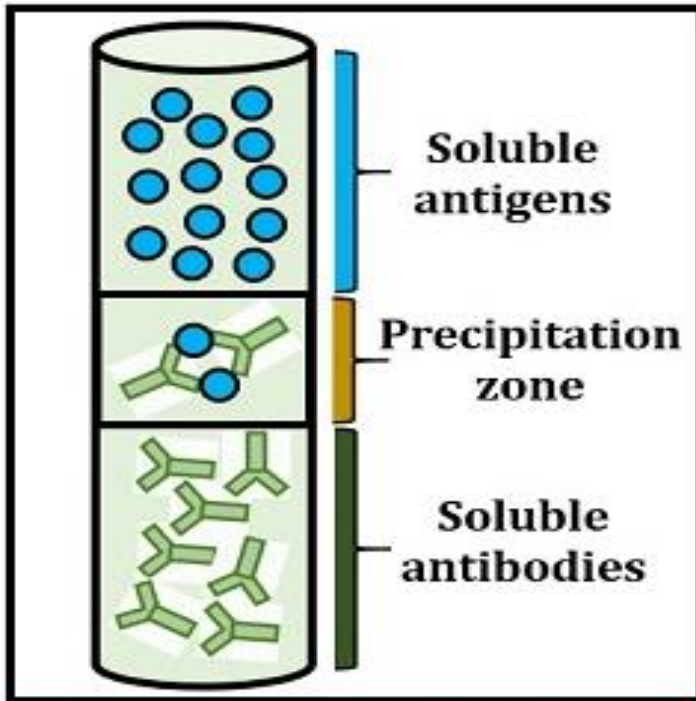
**b. To detect antibodies being made against a specific antigen in the patient's serum. This is called indirect serologic testing.**

Indirect serologic testing is the procedure by which antibodies in a person's serum is made by that individual against an antigen associated with a particular disease are detected using a known antigen.

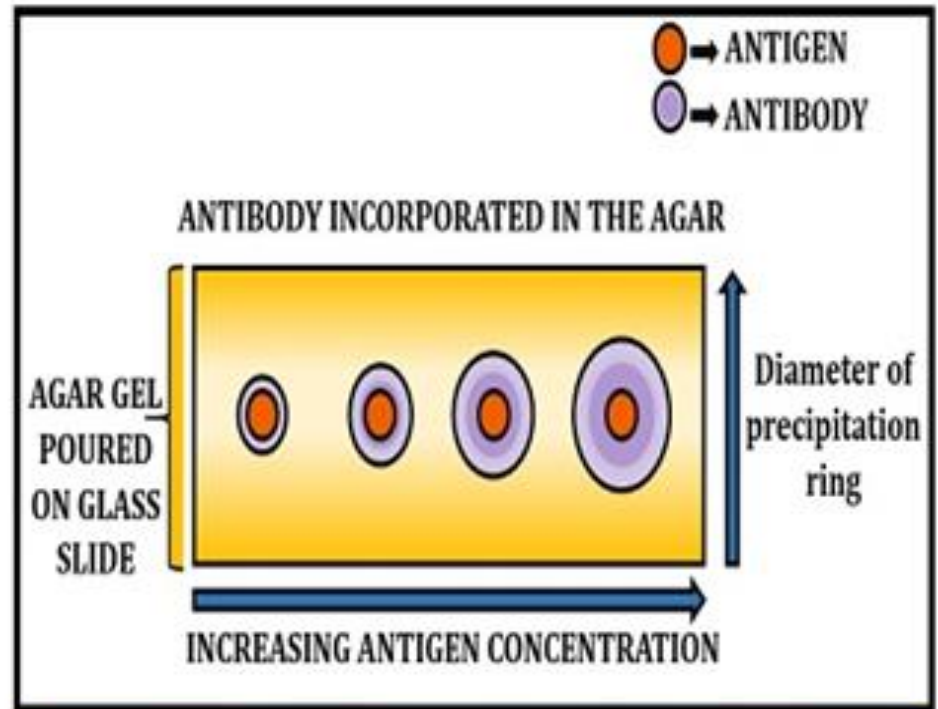
# Techniques for observing in vitro antigen-antibody reactions

**1- A precipitation reaction** is based on the principle of “Antigen-Antibody Reaction”, which occurs at the zone of equivalence.

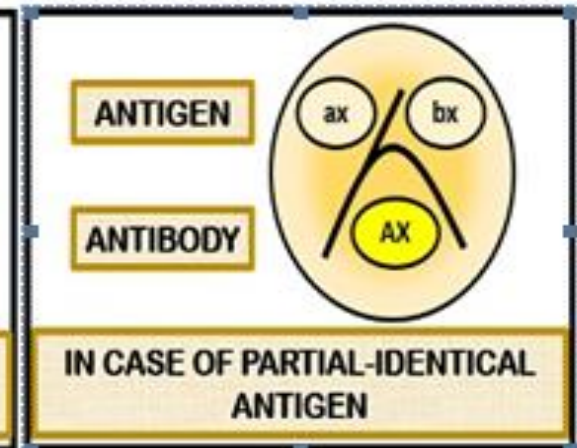
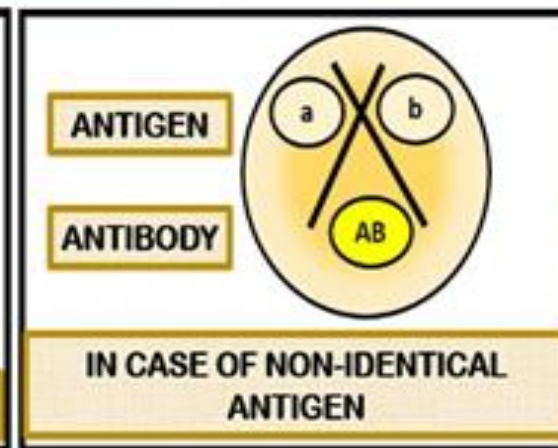
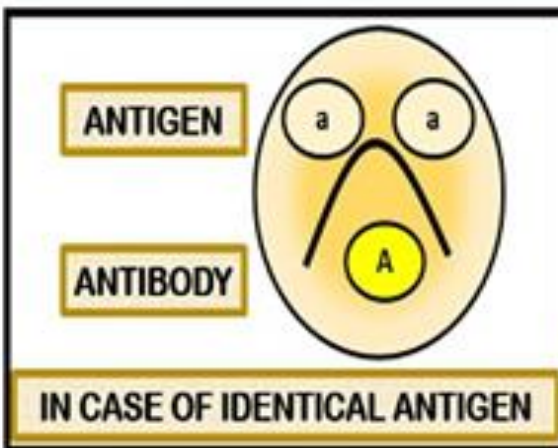
For the appearance of a precipitin ring or band, the reaction may take a **few hours to days**.



**PRECIPITATION RING TEST**

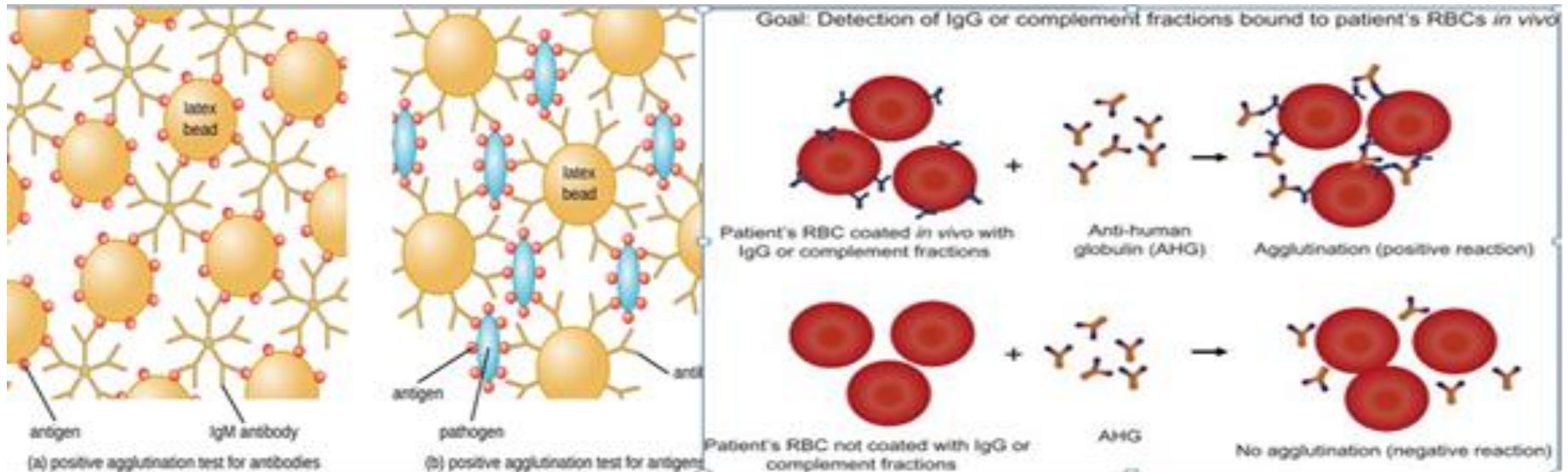


**RADIAL IMMUNODIFFUSION**

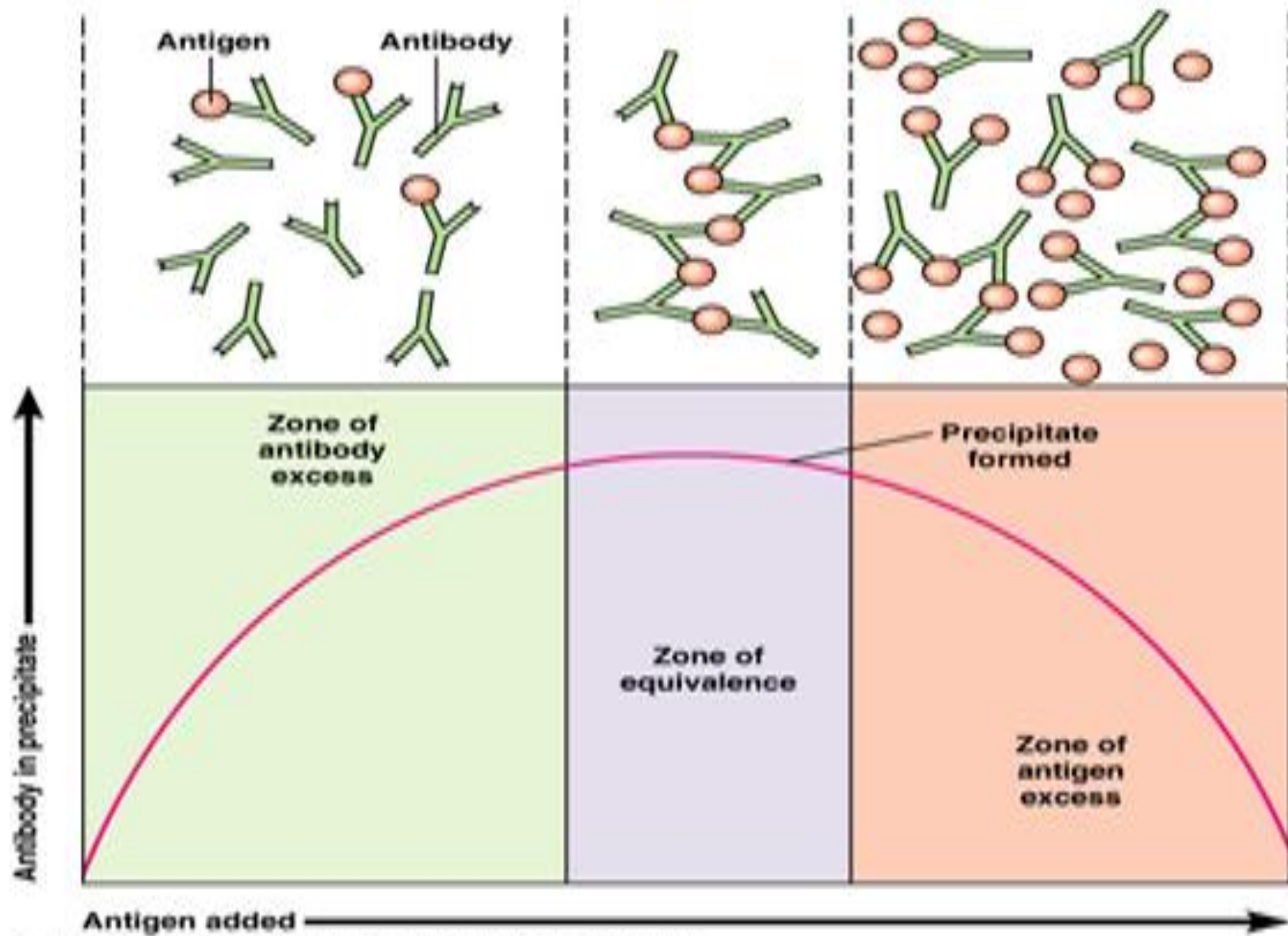


## 2- Agglutination

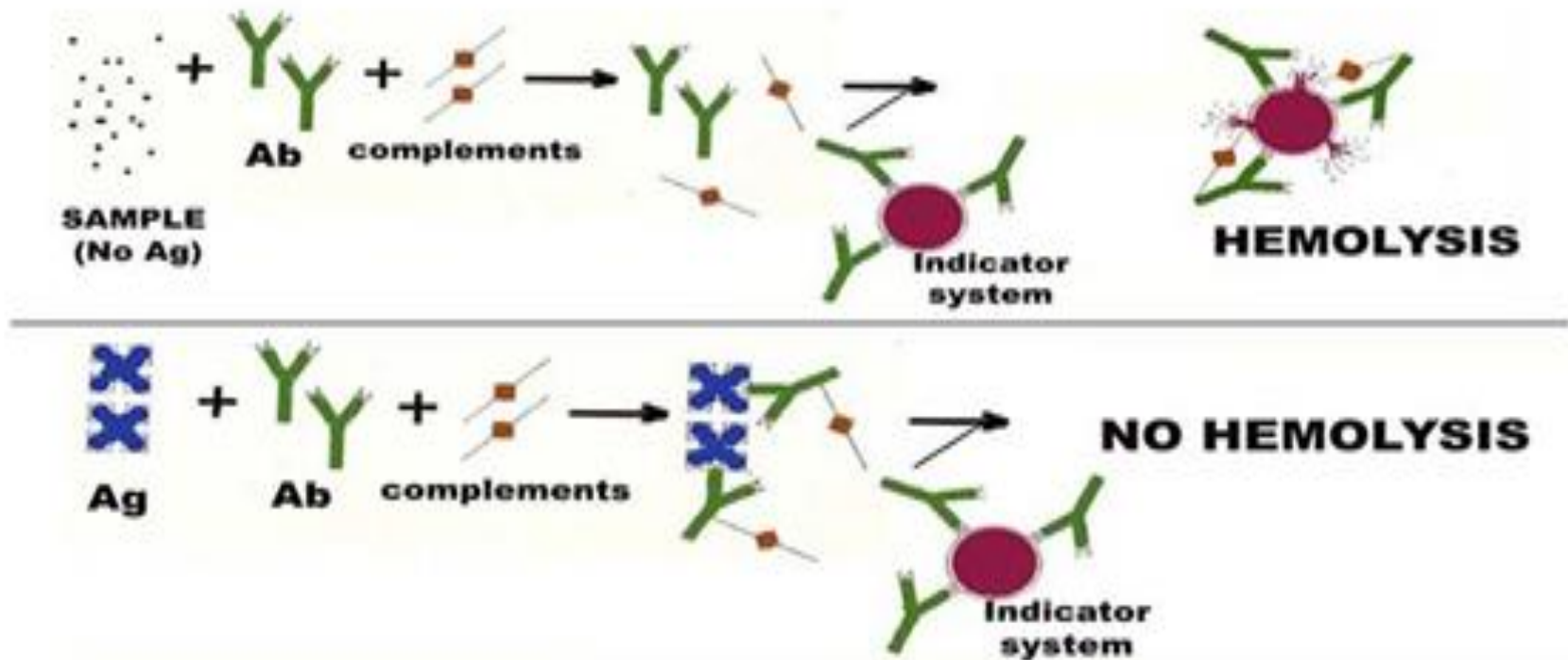
The reaction between a **particulate antigen** as **bacteria, RBC, latex** particles and an antibody results in visible clumping called agglutination. Antibodies that produce such reactions are known as agglutinins.







**3- Complement fixation test** is based on the principle that when antigen and antibodies of the IgM or the IgG classes are mixed, complement is “fixed” to the antigen-antibody complex.

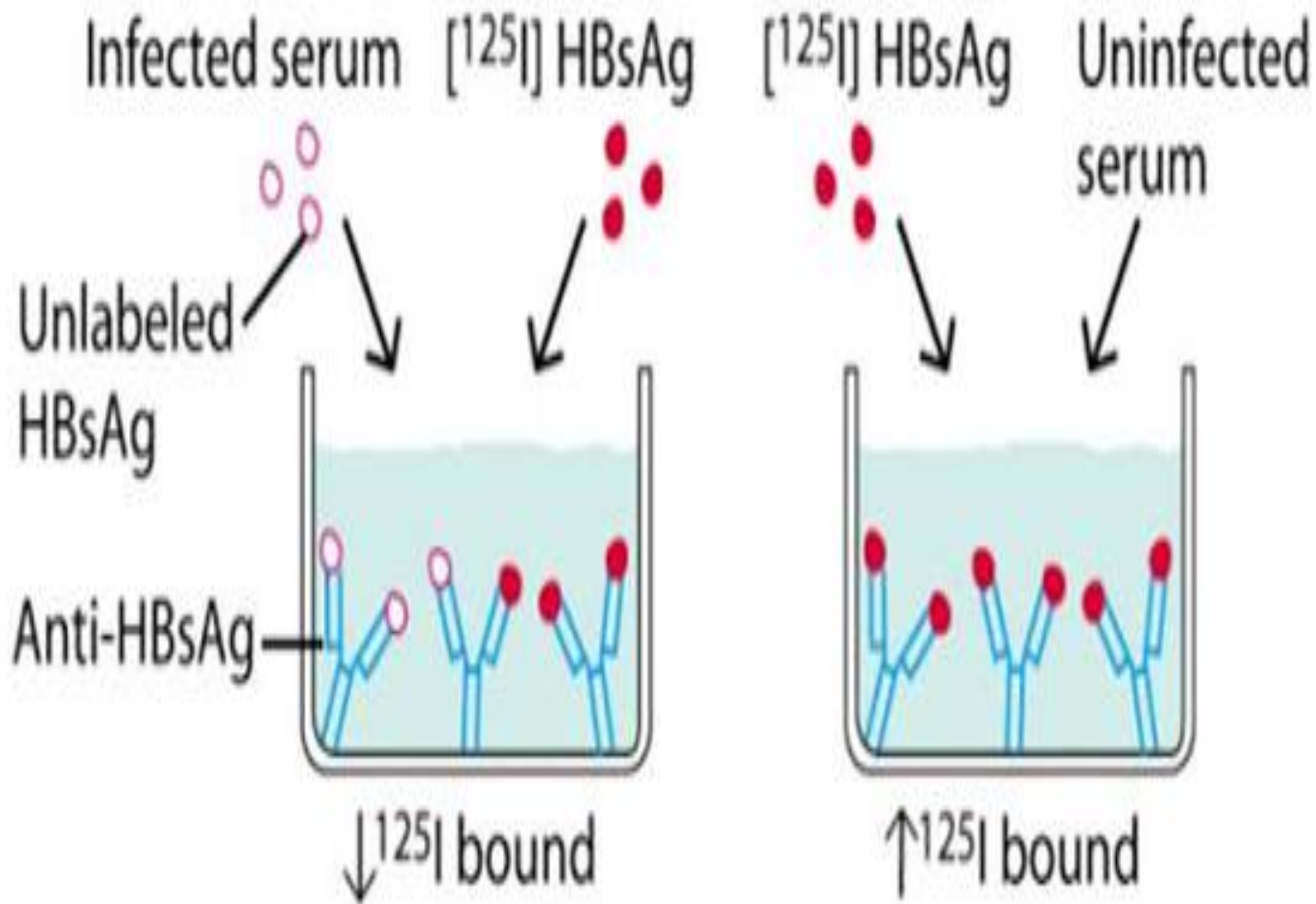


**4- Radioimmunoassay:** A **specific** laboratory test (assay) that uses radiolabeled and unlabeled substances in an immunological (antibody-antigen) reaction

The major **advantages** of RIA, are **higher sensitivity**, easy signal detection, and well-established, rapid assays.

The major **disadvantages** are the health and safety risks. For this reason, RIA has been largely replaced in routine clinical laboratory practice by other serology techniques.

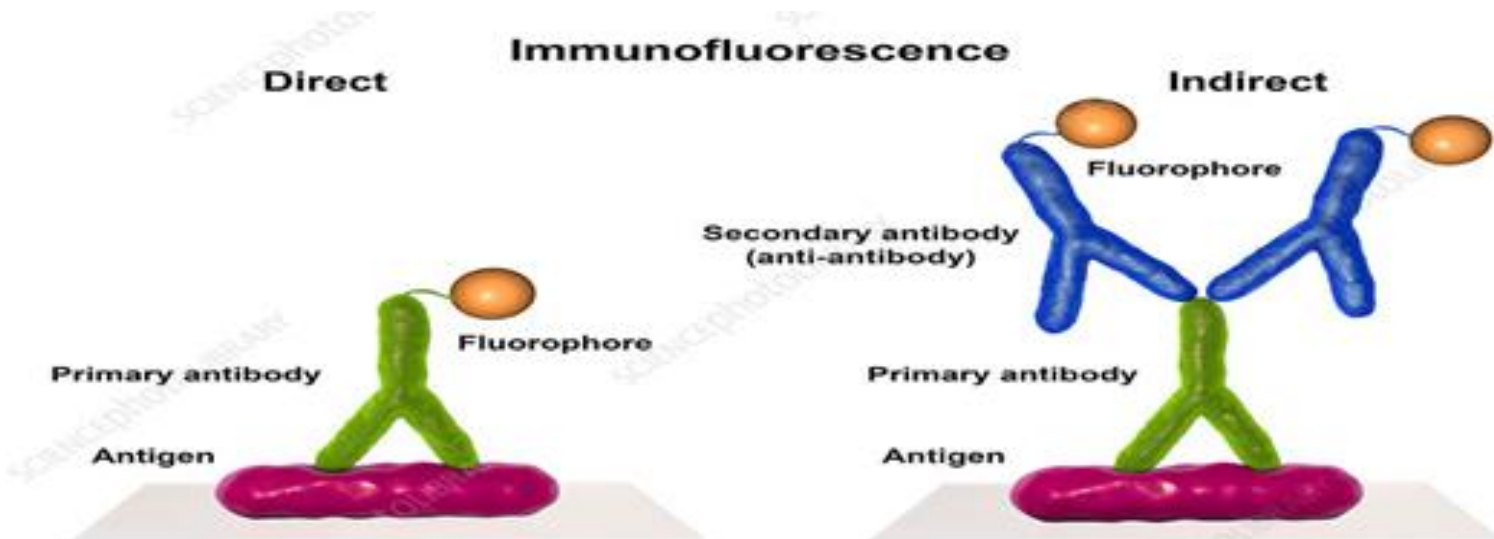




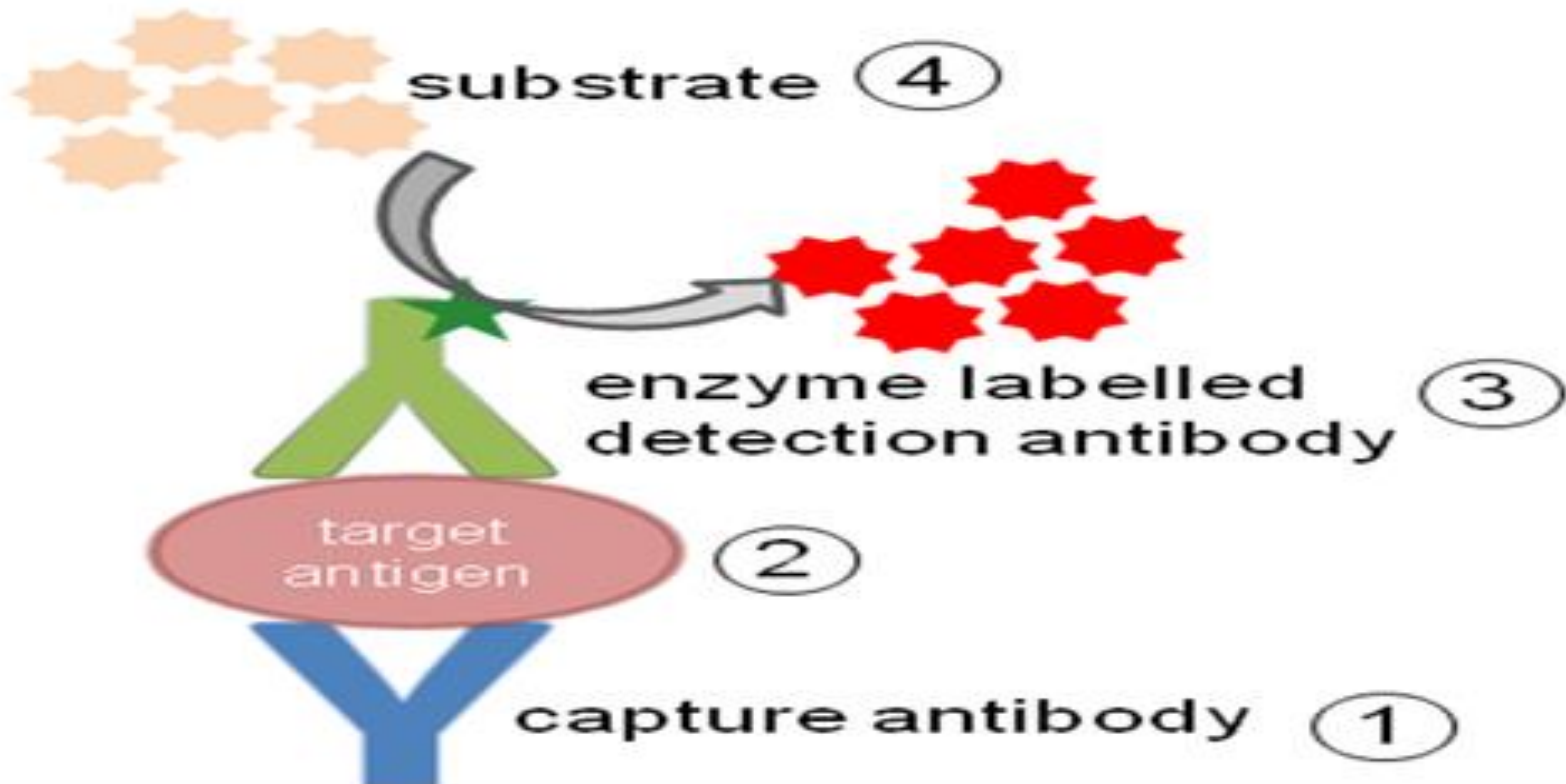
## 5- Fluorescent antibody technique

A fluorescent dye is chemically attached to the known antibodies.

When the fluorescent antibody reacts with the antigen, the antigen will fluoresce when viewed with a fluorescent microscope.



**6- The enzyme-linked immunosorbent assay (ELISA)** is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples.



## **Types of ELISA Assays •**

There are 4 main types of ELISA assays, **Depending on the antigen-antibody combination:**

direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA

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## **7- Western blotting technique:**

In this technique, a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis.

**Southern blot** is used for transferring **DNA**,

**Northern blot** for **RNA** and

**Western blot** for **Protein**.

The proteins thus separated are then transferred or electro transferred onto **nitrocellulose membrane** and are detected using a specific primary antibody and secondary enzyme-labelled antibodies such as horseradish peroxidase (HRP) and substrate.

# DEFENITION

**Immunogen:** a substance that produces a humoral or cell-mediated immune response.

**Antigens** are any substance that binds specifically to an antibody or a T-cell receptor.

**All immunogens are antigens, but all antigens may not be immunogens**

**Hapten** is a molecule that reacts with a specific antibody but is not immunogenic by itself, it can be made immunogenic by conjugation to a suitable carrier like penicillins.

**Polyclonal antibodies;** preparation of known antisera in animals, involves inoculating animals with specific known antigens such as a specific strain of a bacterium.

After the animal's immune responses have had time to produce antibodies against that antigen, the animal is bled and the blood is allowed to clot.

The resulting liquid portion of the blood is the serum and it will contain antibodies specific for the injected antigen.

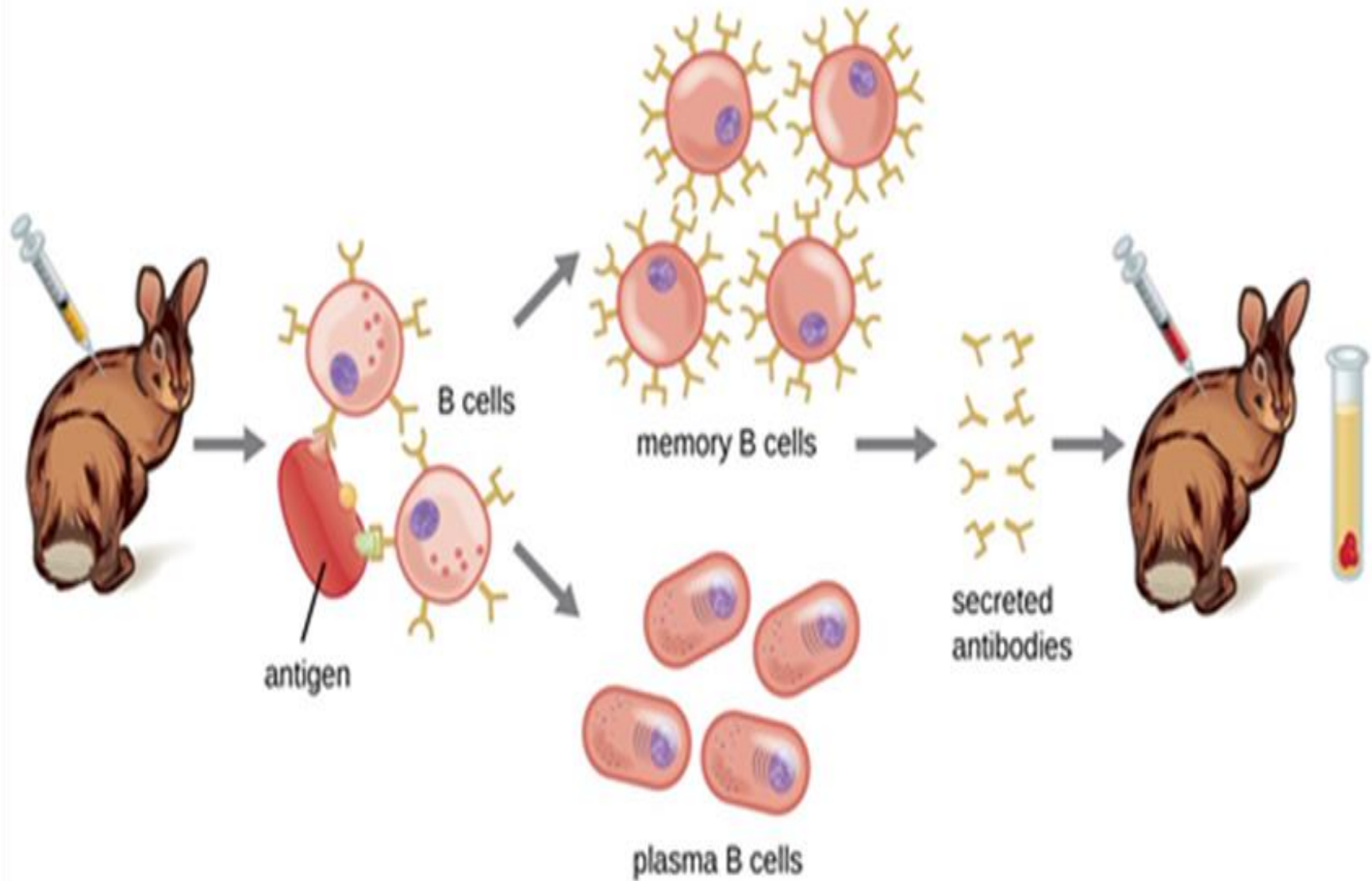


1 Inject antigen into rabbit.

2 Antigen activates B cells.

3 Plasma B cells produce polyclonal antibodies.

4 Obtain antiserum from rabbit containing polyclonal antibodies.



Because most antigens are complex structures with multiple epitopes, they result in the production of multiple antibodies in the lab animal. This so-called **polyclonal antibody** response .

An adjuvant, which is a chemical that provokes a generalized activation of the immune system that stimulates greater antibody production, is often mixed with the antigen prior to injection.

Antiserum obtained from animals will contain antibodies against the antigen artificially introduced in the laboratory, and contain antibodies to any other antigens to which the animal has been exposed during its lifetime. For this reason, antisera must first be “purified” to remove other antibodies before using the antibodies for research or diagnostic assays.

**Monoclonal antibody technique:** the antibodies derived from a single clone of plasma cell; all having the same antigen specificity, produced against a single epitope of an antigen.

In this technique, an animal is injected with the specific antigen for the antibody required. After the appropriate time for antibody production, the animal's spleen is removed.

The spleen is rich in plasma cells and each plasma cell produces only one specific type of antibody. However, plasma cells will not grow artificially in cell culture.

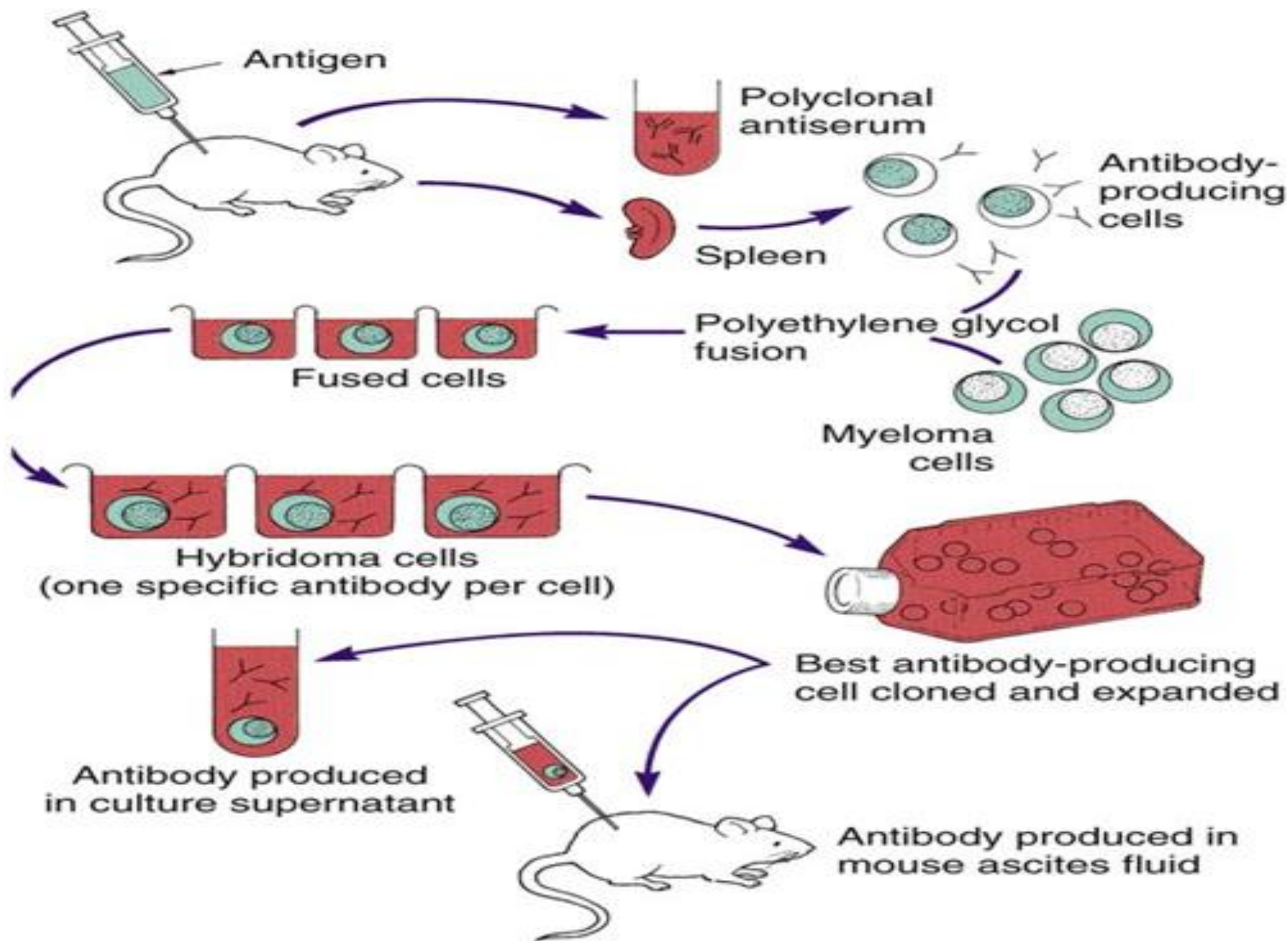
Therefore, a plasma cell producing the required antibody is fused with a myeloma cell, a cancer cell from bone marrow which will grow rapidly in cell culture, to produce a hybridoma cell.

fused hybridoma cells survive in the selective medium and can be recognized by their ability to grow indefinitely in the medium.

when only one clone of B cell is stimulated by a single epitope of an antigen and then is allowed to proliferate and produce antibodies; such antibodies are referred to as monoclonal antibodies (mAb).

Monoclonal antibodies are produced by Hybridoma technique, developed by G Kohler and C Milstein (1975), for which they were awarded Nobel Prize in 1984.

Monoclonal antibodies are now used routinely in medical research and diagnostic serology and are being used experimentally in treating certain cancers and a few other diseases.



<b>Monoclonal Antibodies</b>	<b>Polyclonal Antibodies</b>
<b>Expensive production</b>	<b>Inexpensive production</b>
<b>Long production time</b>	<b>Rapid production</b>
<b>Large quantities of specific antibodies</b>	<b>Large quantities of nonspecific antibodies</b>
<b>Recognize a single epitope on an antigen</b>	<b>Recognize multiple epitopes on an antigen</b>
<b>Production is continuous and uniform once the hybridoma is made</b>	<b>Different batches vary in composition</b>
<b>Monoclonal Antibodies</b>	<b>Polyclonal Antibodies</b>



Human antibodies are classified into five **isotypes** (IgM, IgD, IgG, IgA, and IgE) according to their heavy chain (Mu, Delta, Gamma, Epsilon, or Alpha) which provide each isotype with distinct characteristics and roles.

**Allotypes** represent the genetically determined differences in antibodies between people. Allotypes are used for paternity testing.

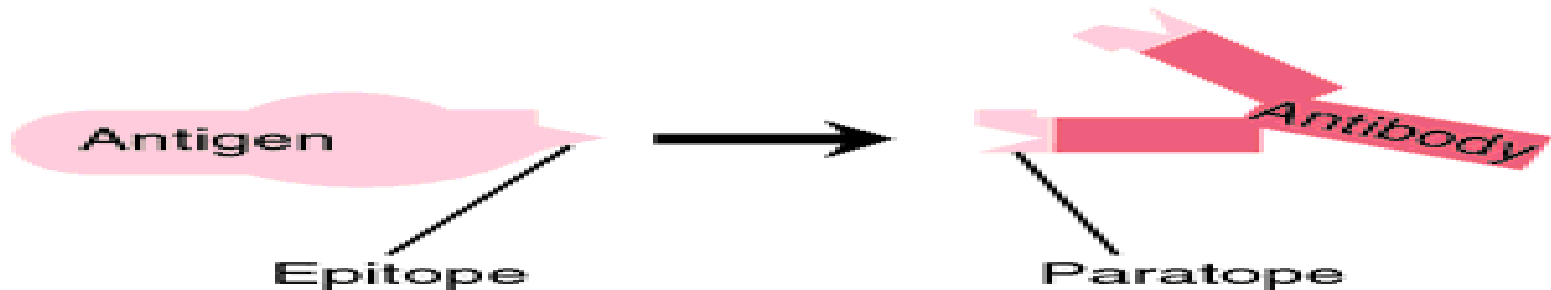
**Idiotypes** are antibodies that recognize different specific epitopes.

Through the end of the variable region; so it known as the set of genetic determinants of an individual; a set of antigen-binding sites which characterizes the antibodies produced by a particular clone of antibody-producing cells.

**Paratope** is a part of an antibody molecule composed of the variable regions of both the light and heavy chains that combine with the antigen.

**Epitope** ( antigenic determinant ) a site on the surface of an antigen molecule to which a single antibody molecule binds;

generally an antigen has several or many different antigenic determinants and reacts with many different antibodies.



**In a primary immune response,**

B cells are stimulated by antigen, become activated, and differentiate into antibody-secreting cells that produce antibodies specific for the eliciting antigen.

**A secondary immune response** is elicited when the same antigen stimulates memory B cells, leading to the production of greater quantities of specific antibodies that are produced in the primary response.

Secondary Immune Response	Primary Immune Response	Characteristics	S.N.
Secondary Immune Response is the reaction of the immune system when it contacts an antigen for the second and subsequent times.	Primary Immune Response is the reaction of the immune system when it contacts an antigen for the first time.	Definition	1
Appears mainly in the bone marrow and then, in the spleen and lymph nodes.	Appears mainly in the lymph nodes and spleen.	Appearance	2
This occurs in response to the second and subsequent exposure to the <b>same</b> antigen.	This occurs in response to the primary contact of the antigen.	Occurrence	3
The antibody level reaches its peak in 3-5 days.	The antibody level reaches its peak in 7-10 days.	Antibody Peak	4
High affinity to their antigens.	Low affinity to their antigens.	Affinity of Antibody	5
Memory B cells	Naive B cells and T cells	Responding Cells	6
Only thymus-dependent antibodies are involved in the secondary immune response.	Both thymus-dependent and thymus-independent antibodies are involved in the primary immune response.	Antibodies	7
Short (1-4 days)	Long (4-7 days)	Lag Phase	8
A large amount of IgG, a small amount of IgM, IgA, and IgE are produced during the secondary immune response.	A large amount of IgM and a small amount of IgG are produced during the primary immune response.	Types of Antibodies	9
100-1000 times more antibodies are produced in the secondary immune response.	Few antibodies are produced in the primary immune response.	Amount of Antibody	10
The secondary immune response is stronger.	The primary immune response is usually weaker than secondary immune response.	Strength of the Response	11
The antibody level tends to remain high for longer.	Antibody level declines to the point where it may be undetectable.	Antibody level	12

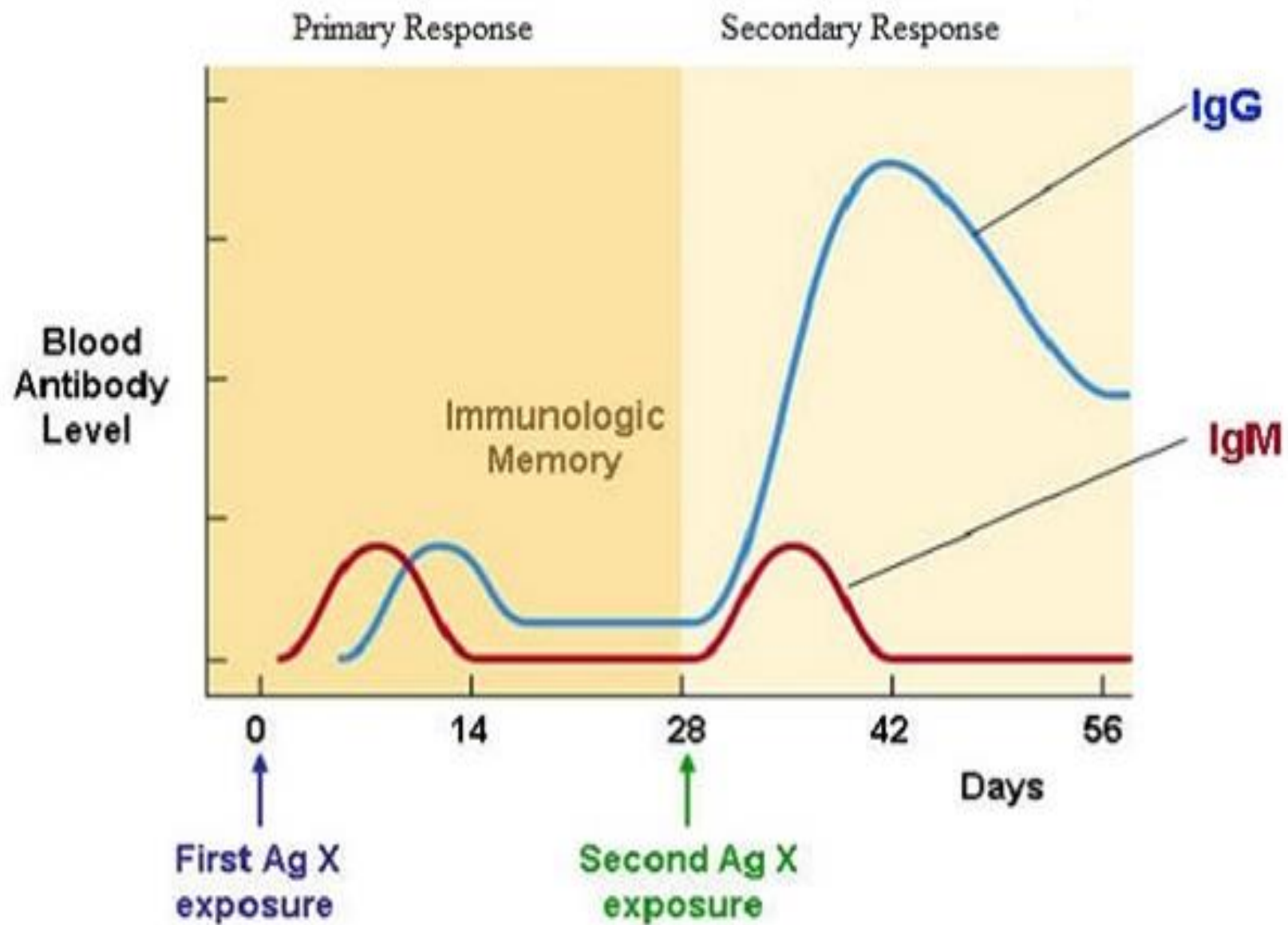


Fig. Immune Response and Secretion of antibodies

**Immunization** is the process whereby a person is made immune or resistant to an infectious disease, typically by the administration of a vaccine against subsequent infection or disease.

**Vaccination** is a treatment with a vaccine to produce immunity against disease; **inoculation** (this term is often used interchangeably with vaccination).  
Vaccine, suspension of weakened, killed, or fragmented microorganisms or toxins or of antibodies or lymphocytes that is administered primarily to prevent disease.