# Department of Biotechnology Medical

**2025-2026**

## Sera and Vaccine

## Stage (Third)

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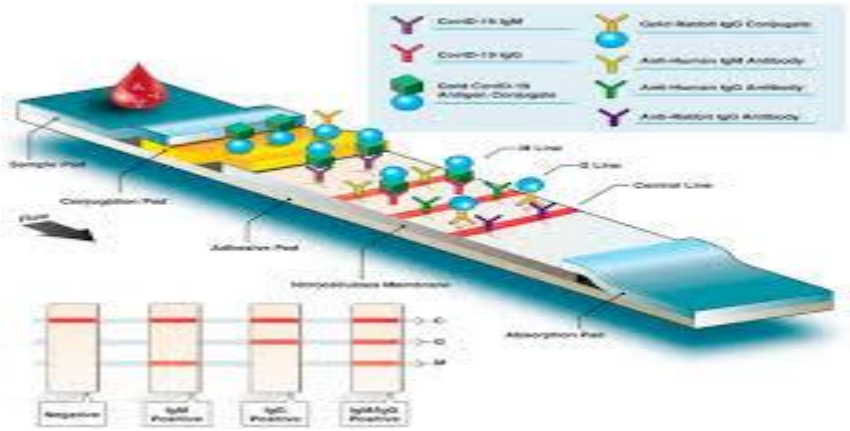

**Serological diagnostic methods**

**By**

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## 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.

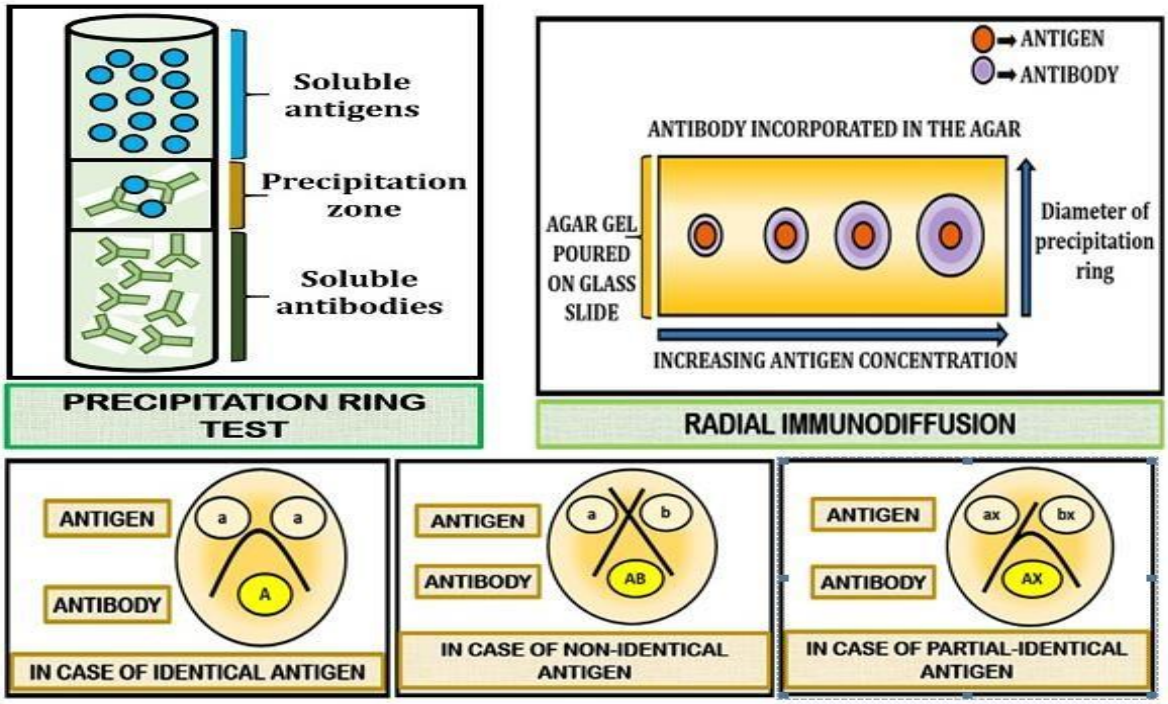
**b. To detect antibodies being made against a specific antigen in the patient's serum.** This is called **indirect serologic testing**.

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

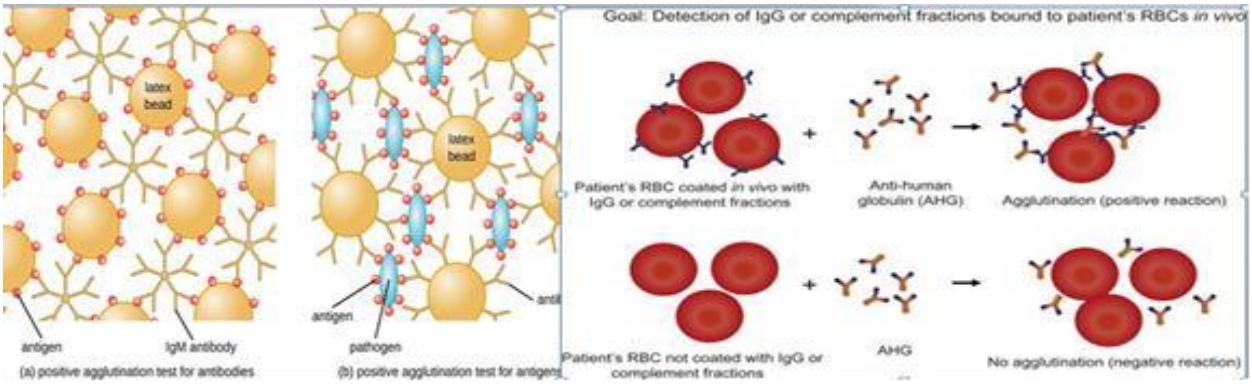
1- **A precipitation reaction** is based on the principle of "Antigen-Antibody Reaction", which occurs at the zone of equivalence.

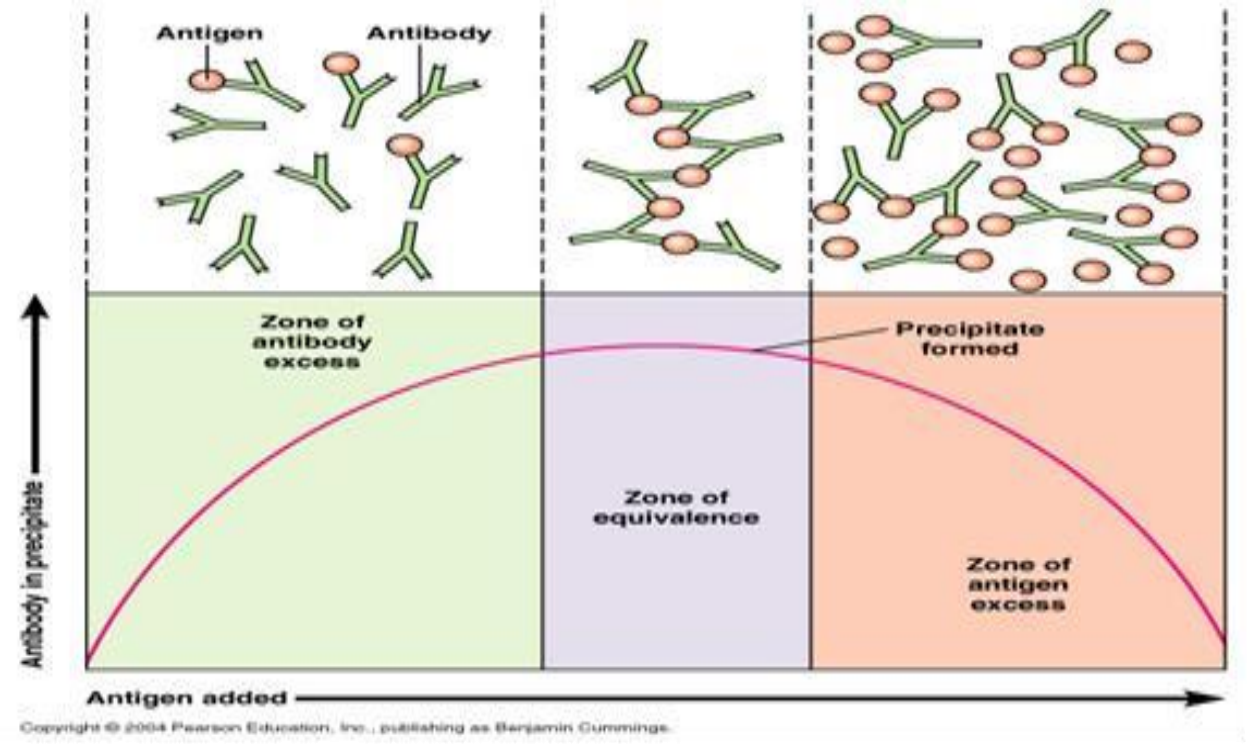
For the appearance of a precipitin ring or band, the reaction may take a **few hours** to **days**.



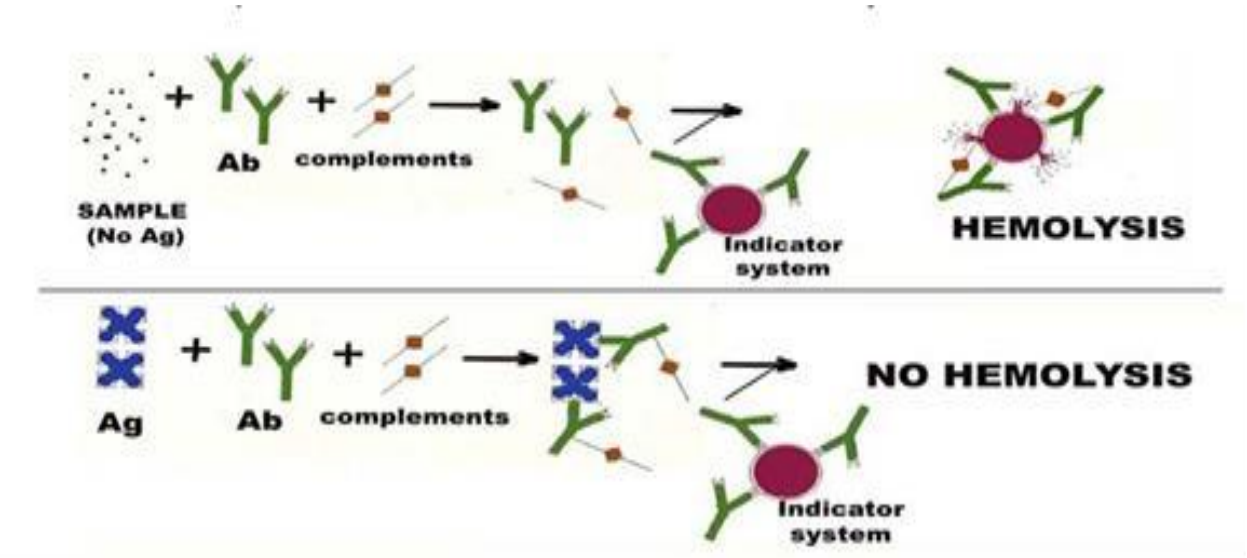

## 2- Agglutination

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 andantibodies 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-

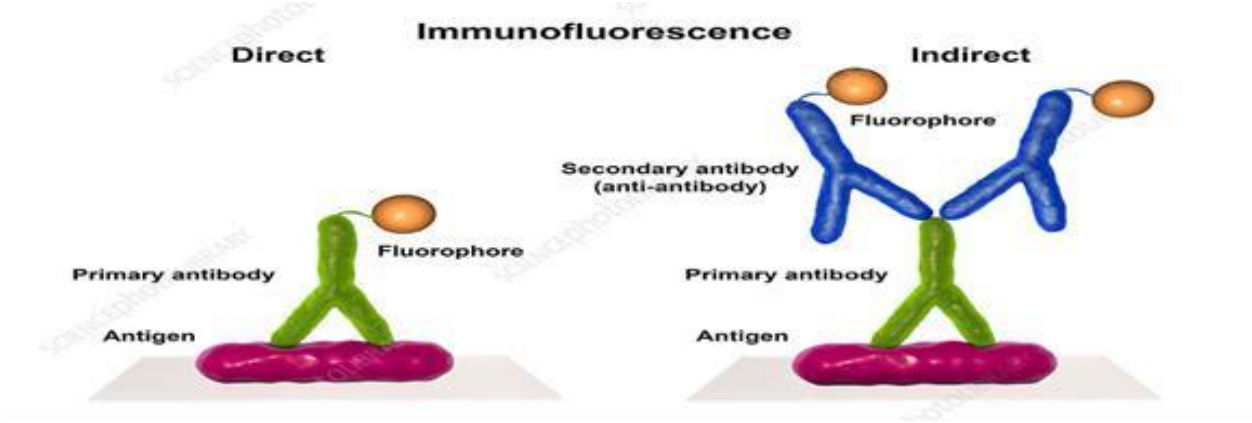
established, rapid assays.

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.

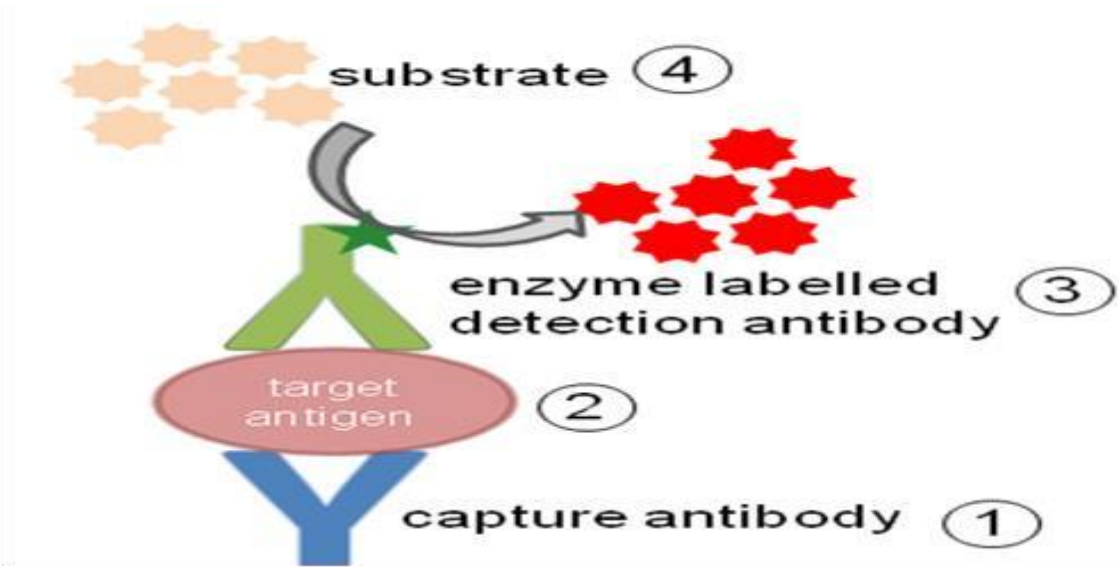
**5- Fluorescent antibody technique**

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

**7- Western blotting technique:**

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

electrophoresis.

**S**outhern blot is used for transferring **D**NA, **N**orthern blot for **R**NA and **W**estern blot for **P**rotein.

The proteins thus separated are then transferred or electro transferred onto **nitrocellulose membrane** and are detected using a specific primary antibody and secondary enzymelabelled 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**

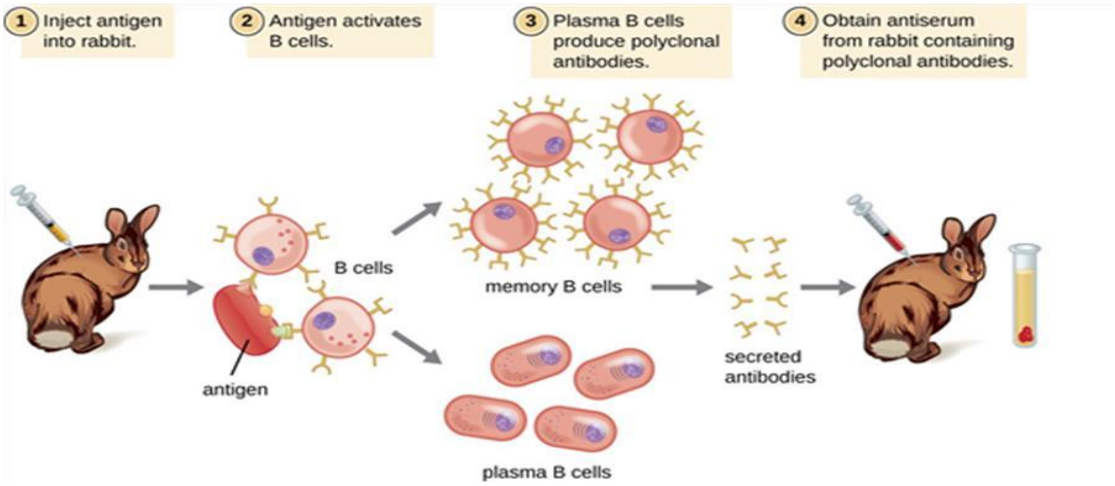
**Hapten** is a molecule that reacts with a specific antibody but is not immunogenic by it self, it can be made immunogenic by conjugation to a suitable carrier like penicillins.

**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.




Because most antigens are complex structures with multiple epitopes, they result in the production of multiple antibodies in the lab animal.

This socalled **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.

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

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.