

Immune Response to Infectious Diseases

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Lesson objectives

After the end of this class, the students will be able to

- ✓ Discuss the immunity for viruses, bacteria, fungi and parasites
- ✓ Distinguish the types of immunity against those pathogens
- ✓ Describe type of immunity for each pathogen

1) Immune response to viral infections

- Viruses are small segments of **nucleic acid** with a **protein** or **lipoprotein** coat
- They require host resources for their **replication**
- Since viruses are **obligate intracellular** organisms, their very existence is threatened /endanger/ if they are destroyed by the **immune system** or by the **death** of their host

- Because of these opposing factors, both viruses and their hosts have been subjected to rigorous/hard/selection and adaptation
- Viruses are selected for their ability to evade the host's immune responses, while at the same time animals are selected for **resistance** to **virus-induced disease**
- Viruses that are **eliminated** before they **replicate** cannot spread

- Hosts **eliminated** by **viruses** can no longer serve as hosts
- Among the survival strategies available to viruses are:
 - 1) A **long latency** period before severe illness or death occurs. E.g. HIV
 - 2) Facile /superficial/ transmission, in which infection is efficiently transferred during even a short **acute illness**. E.g. **Influenza** and **smallpox viruses**

- A number of specific **immune effector mechanisms**, together with **nonspecific defense mechanisms**, are called into play to prevent or eliminate **viral infection**

I. Innate immunity

- Rapid, powerful **innate immune** responses limit many viral infections by **inhibiting viral replication**
- ✓ Type **I interferon's** (IFN- α and IFN- β) are especially important to **viral resistance**.
- ✓ They are produced by **virus infected cells, macrophages, monocytes and fibroblasts**

- ✓ NK cells kill **virus infected cells**.
- ✓ NK cell cytotoxicity is stimulated by **Type I interferons** and **IL-12**.
- ✓ **NK cells** produce **IFN- γ** (Type II interferon) and this too has a direct **antiviral effect**

- ✓ **Lysozyme** can destroy several viruses, as can many intestinal enzymes and bile
- ✓ **Collectins** bind to **viral glycoproteins** and block **virus interaction** with **host cells**
- ✓ **Defensins** from **leukocytes** and **mucosal epithelial cells** play a dual role in **antiviral defenses** since they can act both on the **virus** and on the **host cells**
- ✓ Cells invaded by viruses may undergo **premature apoptosis**, thus preventing successful **viral invasion** and replication

ii) Acquired immunity

A. Antibody –mediated immunity

- Viral capsids are antigenic and it is against these and the envelope proteins that antiviral immune responses are largely mounted

Antibodies can prevent cell invasion by:

- ✓ Blocking the adsorption of **virions** to **target cells**
- ✓ Stimulating **phagocytosis** of viruses
- ✓ Triggering **complement-mediated** virolysis
- ✓ Causing viral clumping and so reducing the number of infectious units available for cell invasion

- Antibodies are also directed against **viral antigens** expressed by infected cells. As a result, these infected cells may also be destroyed.
- E.g. NCD, rabies, bovine viral diarrhea, infectious bronchitis of birds, etc
- Antibodies may kill infected cells by complement mediated cytolysis or by antibody-dependent cell-mediated cytotoxicity (ADCC).

- These **cytotoxic cells** include **lymphocytes, macrophage, and neutrophils** with **Fc receptors** through which they can bind to **antibody-coated target cells**
- The **immunoglobulins** that **neutralize viruses** include **IgG** and **IgM** in **serum**, and **IgA** in **secretions**.
- IgE may also play a **protective role**

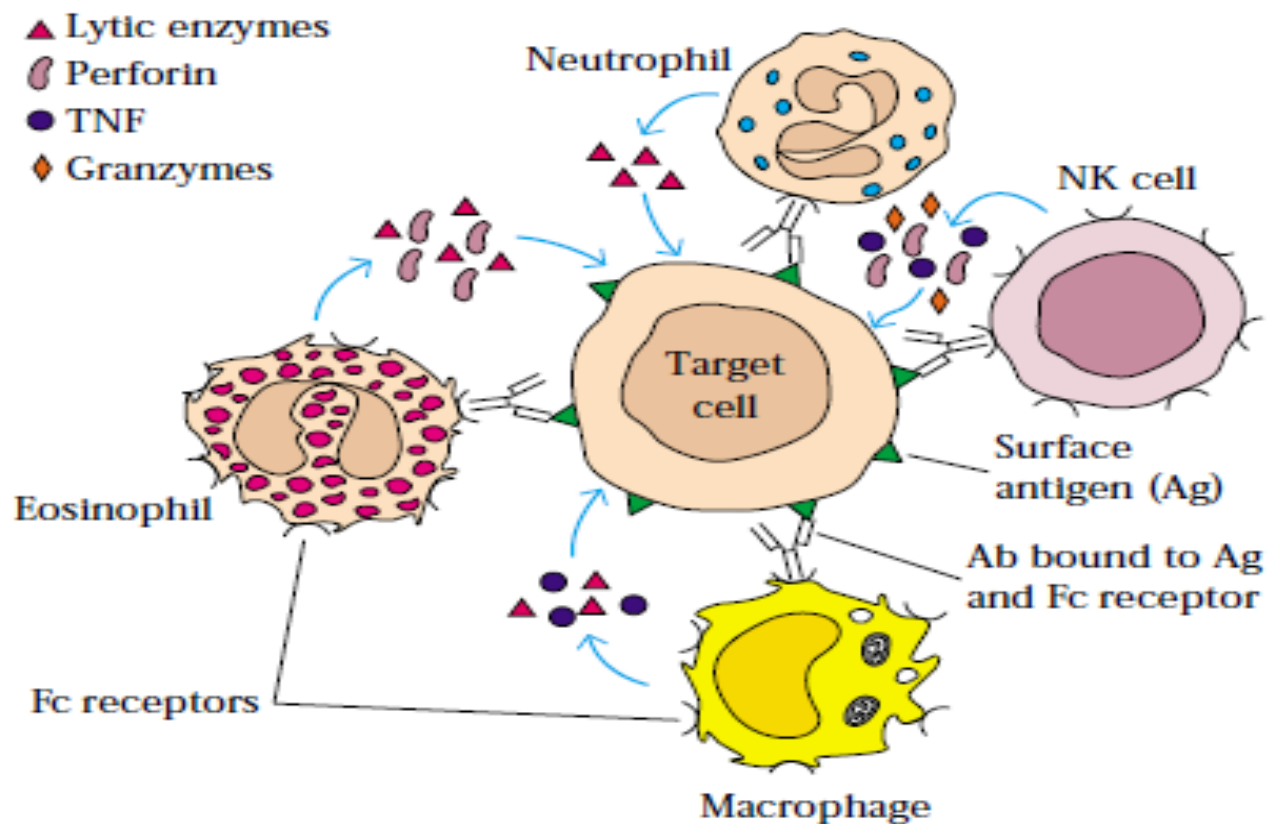


FIGURE 14-15 Antibody-dependent cell-mediated cytotoxicity (ADCC). Nonspecific cytotoxic cells are directed to specific target cells by binding to the Fc region of antibody bound to surface antigens on the target cells. Various substances (e.g., lytic enzymes, TNF, perforin, granzymes) secreted by the nonspecific cytotoxic cells then mediate target-cell destruction.

B) Cell-Mediated Immunity

- **Antibodies** are not usually able to eliminate the virus once infection has occurred
- Once an infection is established, **cell-mediated immune mechanisms** are most important in host defense.
- In general, CD_8^+ T_C cells and CD_4^+ T_H^1 cells are the main components of **cell-mediated antiviral defense**
- Activated T_H^1 cells produce a number of **cytokines**, including **IL-2**, **IFN- γ** and **TNF**, that defend against viruses either directly or indirectly.

- **IFN- γ** acts directly by inducing an **antiviral state** in cells.
- **IL-2** acts indirectly by assisting in the recruitment of **CTL precursors** into an effector population.
- Both IL-2 and IFN- γ activate **NK cells**, which play an important role in host defense during the first days of many viral infections until a specific CTL response develops

- In most viral infections, specific CTL activity arises within 3–4 days after infection, peaks by 7–10 days, and then declines.
- Within 7–10 days of primary infection, most virions have been eliminated, paralleling the development of CTLs.

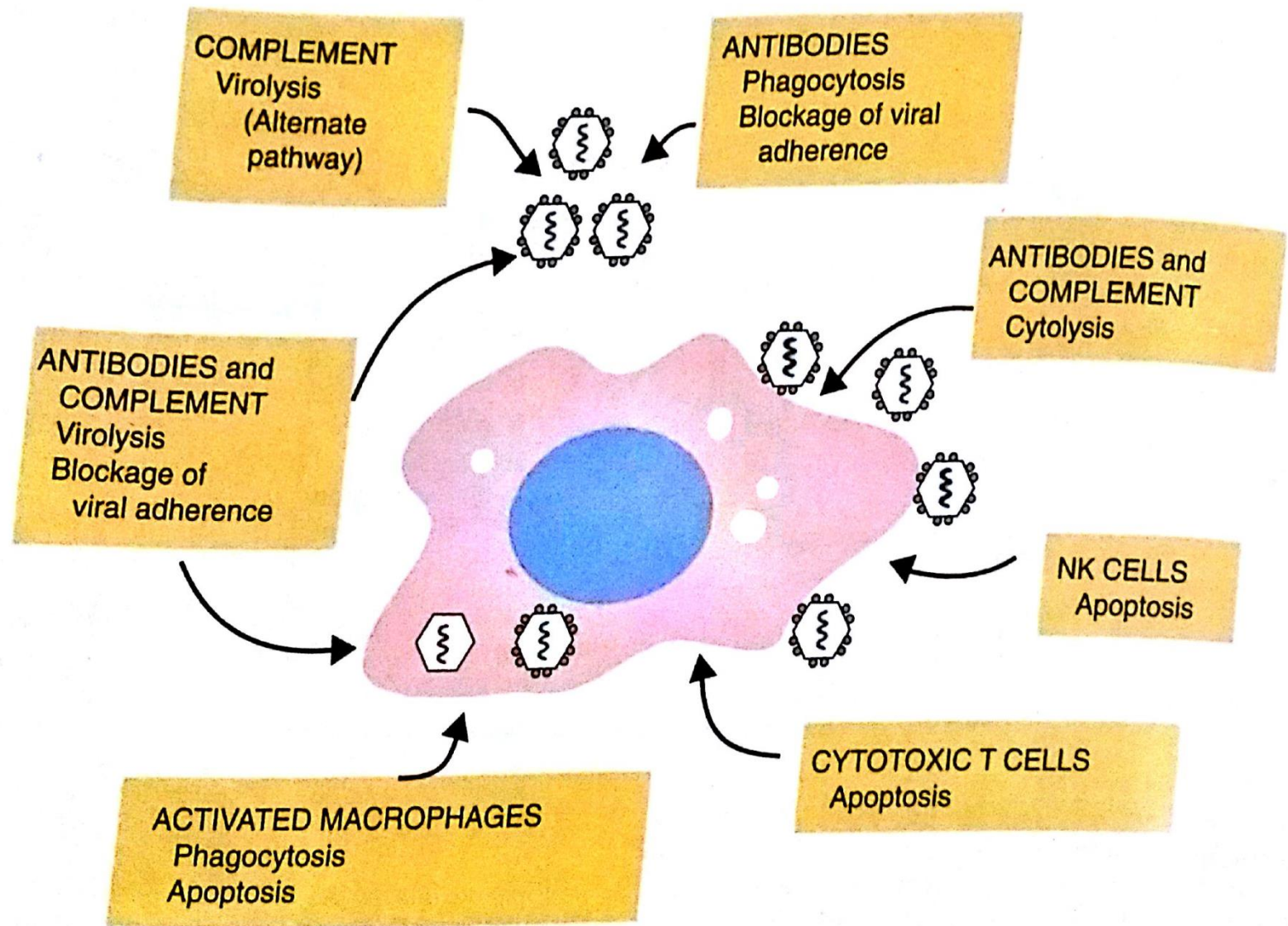


FIGURE 23-6 ■ The ways in which the immune system can protect the body against viruses.

2) Immune response to bacterial infection

- Antimicrobial immunity consists of an **early innate response** followed by a more sustained **adaptive response**
- Recognition of invading bacteria through **toll-like receptors** (TLRs) and other receptors induces, **inflammation, cytokine release** and **complement activation**
- If this is **insufficient** to eliminate the invaders, **acquired** immune mechanisms take over

- Dendritic cells and macrophages ingest invading bacteria and initiate acquired immunity by secreting cytokines and triggering both T and B cells responses.

- TLRs play a role in recognizing invading microbes and in triggering inflammation
- These receptors are expressed on (sentinel cells) macrophages and mast cells, as well as on dendritic cells, eosinophils and epithelial cell in the respiratory tract and intestine

- Pathogen Associated Molecular Patterns include the Lipopolysaccharides of gram negative bacteria, the lipoteichoic acids of gram positive bacteria.
- The mannans of yeasts and the glycolipids of mycobacteria as well as bacterial DNA.
- All of these molecules are restricted to microorganism and are not found in animal tissue

- Bacterial DNA-is a significant stimulator of innate immunity. It differ from eukaryotic DNA in that it contains a large proportion of the dinucleotide cytosine–guanosine (CpG).
- In addition while the cytosine in eukaryotic DNA is normally methylated this is not the case in bacterial DNA
- Thus unmethylated CpG dinucleotides can trigger innate immune response

- There are five basic mechanism by which the acquired immune responses combat bacterial infections
 - i) Neutralization of toxins or enzymes by antibody
 - ii) Killing of bacteria by antibodies and complement
 - iii) Opsonization of bacteria by antibodies and complement, resulting in their phagocytosis and destruction

- iv) Destruction of intracellular bacteria by activated macrophages
- v) Direct killing of bacteria by cytotoxic T cells and natural killer cells

❖ Immune response to extracellular bacteria

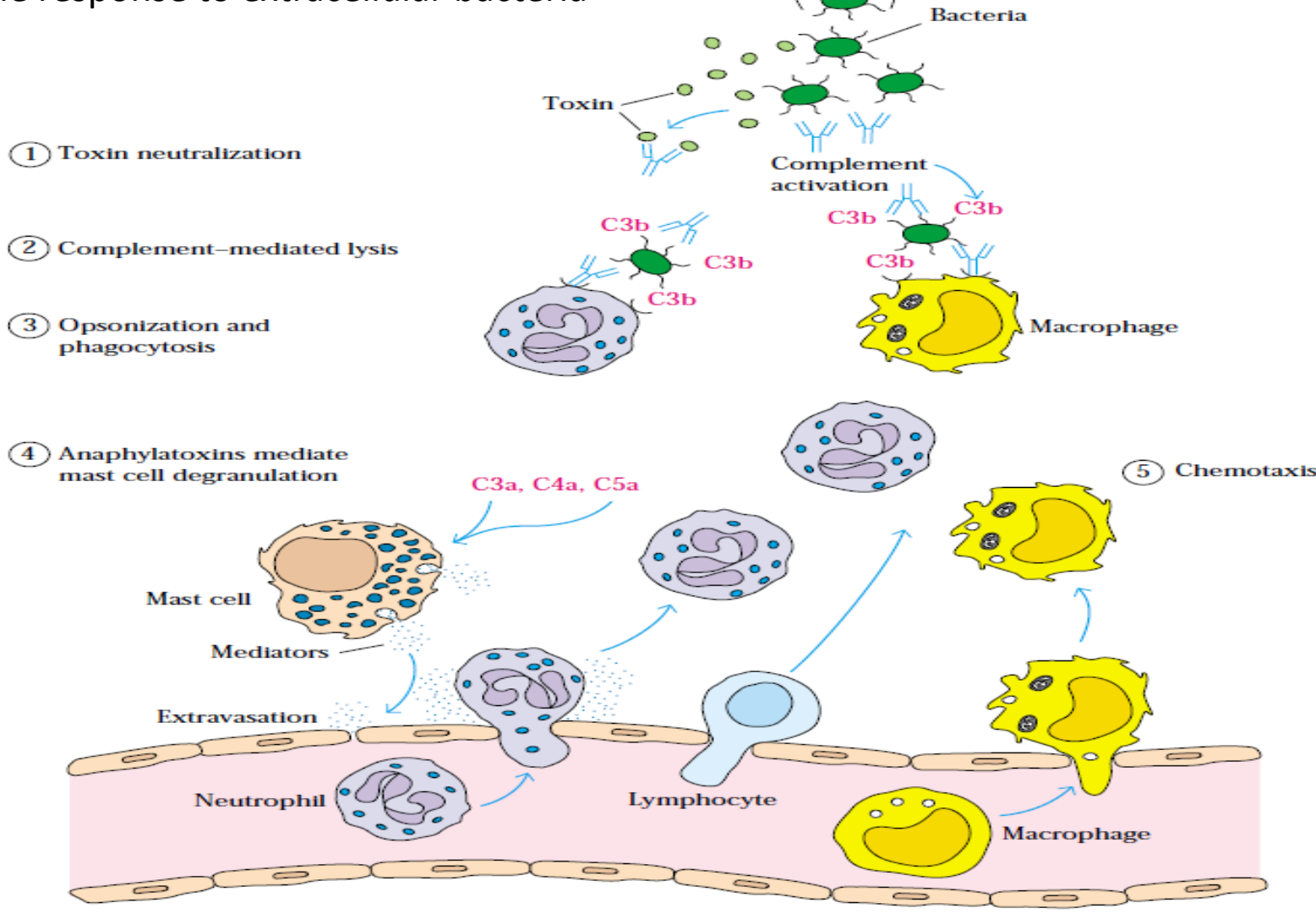


FIGURE 17-8 Antibody-mediated mechanisms for combating infection by extracellular bacteria. (1) Antibody neutralizes bacterial toxins. (2) Complement activation on bacterial surfaces leads to complement-mediated lysis of bacteria. (3) Antibody and the complement split product C₃b bind to bacteria, serving as opsonins to increase phagocytosis. (4) C3a, C4a, and C5a, generated by antibody-initiated complement activation, induce local mast cell degranulation, releasing substances that mediate vasodilation and extravasation of lymphocytes and neutrophils. (5) Other complement split products are chemotactic for neutrophils and macrophages.

❖ Immune response to intracellular bacteria

- While innate immunity is not very effective against intracellular bacterial pathogens, intracellular bacteria can activate NK cells, which, in turn, provide an early defense against these bacteria.
- Intracellular bacterial infections tend to induce a cell-mediated immune response, specifically, delayed type hypersensitivity.

- In this response, cytokines secreted by CD_4^+ T cells are important—notably IFN- γ , which activates macrophages to kill ingested pathogens more effectively

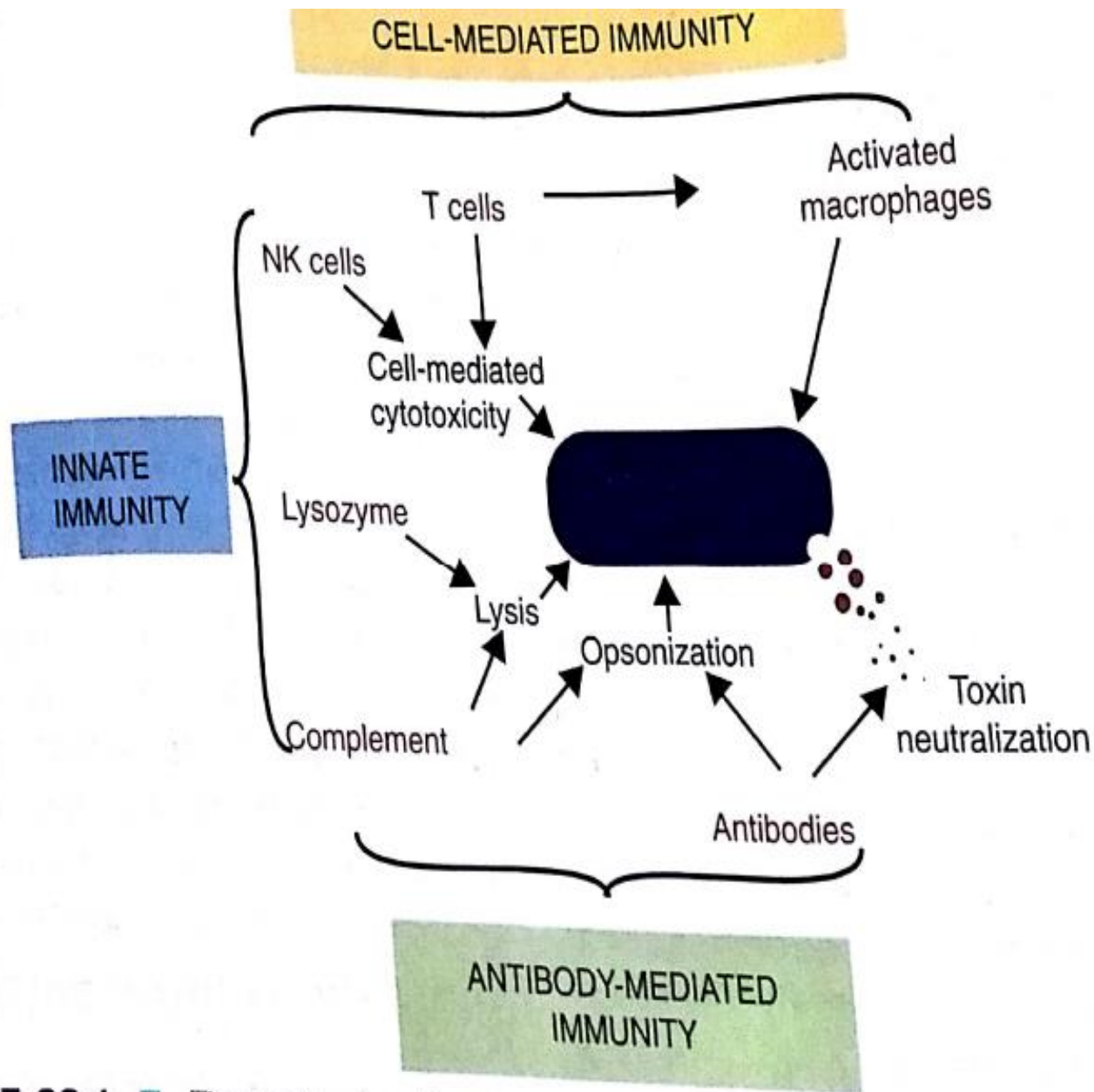


FIGURE 22-1 ■ The mechanisms by which the immune responses can protect the body against bacterial invasion.

3) Immune response to fungal infection

- The barrier of innate immunity control most fungi
- Innate immune mechanism against invasive fungi such as *Candida* spp or *Aspergillus* spp include activation of the alternative pathway of the complement system resulting in attraction of neutrophils and attempts by these neutrophils to ingest the invading hyphae or pseudohyphae

- Neutrophils are also activated by the IL-23/IL-17
- IL-23 activates T_h17 cells.
- The IL-17 produced by T_h17 cells then activates both neutrophils and endothelial cells and promote acute inflammation
- Once established, fungal infections can be destroyed only by Th1-mediated mechanisms

- T_h1 functions primarily in fungal infections by activating macrophages and by promoting epidermal growth and keratinization
- Some T and NK cells can exert a direct cytotoxic effect on yeast such as *Cryptococcus neoformans* and *Candida albicans*

4) Immune response to parasitic infection

a) Immunity to Protozoa

- The mechanism of innate resistance to protozoa appear to be similar to those of that prevent bacterial and viral invasion.
- Like other invaders, protozoa stimulate both antibody and cell-mediated immune response

- In general, antibodies control the numbers of parasites in blood and tissue fluids, whereas cell-mediated responses are directed largely against intracellular parasites
 - ✓ Serum antibodies directed against protozoan surface antigens may opsonize, agglutinate, or immobilize them
 - ✓ Antibodies together with complement and cytotoxic cells may kill them

- ✓ Some antibodies may inhibit their division
- In babesiosis, the infective stages of the organisms (sporozoites) invade red blood cells.
- Infected red cells incorporate *Babesia* antigens into their membranes.
 - These in turn induce antibodies that opsonize the red cells and cause their removal by phagocytosis

- Infected red cells may also be destroyed by ADCC.
(antibody-dependent cell-mediated cytotoxicity)
- Macrophages and cytotoxic lymphocytes can recognize the opsonizing-antibody complex on the surface of infected erythrocytes
- Cytotoxic T cells may play a role early in infection, when the number of infected erythrocytes is small

- Protective immunity against *Cryptosporidia*, *Eimeria*, *Neospora*, *Plasmodia* and *Toxoplasma* is generally mediated by T_h1 response.
- T_h1 cells secrete $INF-\gamma$, $INF-\gamma$ activates macrophages enabling them to kill the intracellular organism by permitting lysosome-phagosome fusion.

b) Immunity to Helminths

- Although the complement and other factors of natural immune response might contribute to protecting against the helminth infections, specific immune response with the production of antibodies and cytokines is important.

- The Th² cells are producers of cytokines like IL-4, IL-5 and IL-13 which, among their other functions, induce IgE production by B cells and activate the fundamental components in protecting against helminths, namely eosinophils, mastocytes and basophiles, respectively.

- IgE antibodies bind to circulating basophiles or tissue mast cells.
- This induces the release of histamine and other reaction mediators of immediate hypersensitivity leading to the destruction of helminths

- Macrophage and eosinophils with CD23(Fc receptor),
These cells can therefore bind to IgE-coated parasites,
become activated, and kill them
- Th¹ cells responses may be little protective benefits
- Cytotoxic T cells may attack helminths that are
deeply embedded in the intestinal mucosa or
undergoing tissue migration

Hypersensitivity reactions

- Immune system has effector molecules that act to remove antigen by various mechanisms. Generally, these effector molecules induce a localized inflammatory response that eliminates antigen without extensively damaging the host's tissue.
- Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed hypersensitivity or allergy.

Con't..

- Although the word hypersensitivity implies an increased response, the response is not always heightened but may be an inappropriate immune response to an antigen. Hypersensitive reactions may develop in the course of either humoral or cell-mediated responses.

Types of hypersensitivity reactions

I. IgE-Mediated (Type I) Hypersensitivity

- A type I hypersensitive reaction is induced by certain types of antigens referred to as allergens, and has all the hallmarks of a normal humoral response. That is, an allergen induces a humoral antibody response by the same mechanisms as for other soluble antigens, resulting in the generation of antibody-secreting plasma cells and memory cells.

- What distinguishes a type I hypersensitive response from a normal humoral response is that the plasma cells secrete IgE.
- Examples: different allergens: The term allergen refers specifically to non parasitic antigens capable of stimulating type I hypersensitive responses in allergic individuals.

II. Antibody-Mediated Cytotoxic (Type II) Hypersensitivity

- Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell, or it can mediate cell destruction by antibodydependent cell-mediated cytotoxicity (ADCC).

Con't..

- In this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells. Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to bind and phagocytose the antibody-coated cell.

Examples: blood Transfusion Reactions.

III. Immune Complex–Mediated (Type III)

Hypersensitivity

- The reaction of antibody with antigen generates immune complexes. Generally this complexing of antigen with antibody facilitates the clearance of antigen by phagocytic cells.
- In some cases, however, large amounts of immune complexes can lead to tissue-damaging type III hypersensitive reactions.

Con't..

- The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body. When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops. When the complexes are formed in the blood, a reaction can develop wherever the complexes are deposited.

Con't..

- In particular, complex deposition is frequently observed on blood-vessel walls, in the synovial membrane of joints, on the glomerular basement membrane of the kidney, and on the choroid plexus of the brain. The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. The tissue there is injured as a consequence of granular release from the neutrophil.

IV. Delayed type hypersensitivity reaction (type 4)

- When certain antigens are injected into the skin of a sensitized animal, an inflammatory response, taking many hours to develop, may occur at the injection site. This is a T helper cell– mediated response called delayed hypersensitivity.
- Delayed hypersensitivity reactions are classified as type IV hypersensitivity reactions.

- An important example of a delayed hypersensitivity reaction is the tuberculin response—the skin reaction that follows an intradermal injection of tuberculin.
- A different form of type IV hypersensitivity occurs in allergic contact dermatitis. This is a slowly developing inflammatory response that occurs when reactive chemicals bind to skin cells and trigger T cell responses.

Diseases of the immune system

Autoimmunity

- An inappropriate response of the immune system against self-components is termed autoimmunity.
- autoimmune diseases can be divided into organ specific and systemic diseases. The organ-specific diseases involve an autoimmune response directed primarily against a single organ or gland.

Con't..

- The systemic diseases are directed against a broad spectrum of tissues and have manifestations in a variety of organs resulting from cell-mediated responses and cellular damage caused by auto-antibodies or immune complexes.
- Studies with experimental autoimmune animal models have revealed a central role for CD4⁺ TH cells in the development of autoimmunity. In each of the experimentally induced autoimmune diseases, autoimmune T-cell clones can be isolated that induce the autoimmune disease in normal animals.

Con't..

- The relative number of TH1 and TH2 cells appears to play a pivotal role in determining whether autoimmunity develops: TH1 cells promote the development of autoimmunity, whereas TH2 cells appear to block development of autoimmune disease and also block the progression of the disease once it is established.

Con't..

- A variety of mechanisms have been proposed for induction of autoimmunity, including release of sequestered antigens, molecular mimicry, inappropriate class II MHC expression on cells (in some cases stimulated by IFN-, and polyclonal B-cell activation).

- Current therapies for autoimmune diseases include treatment with immunosuppressive drugs, thymectomy, and plasmapheresis for diseases involving immune complexes.
- Other therapies include vaccination with T cells specific for a given autoantigen, administration of synthetic blocking peptides that compete with autoantigen for binding to MHC molecules, treatment with monoclonal antibodies that react with some component specifically involved in an autoimmune reaction, and induction of tolerance to autoantigens by administering them orally.

Immunodeficiency

- If any of the many components of innate or specific immunity is defective because of genetic abnormality, or if any immune function is lost because of damage by chemical, physical, or biological agents, the host suffers from immunodeficiency.
- Any defect in either the innate or adaptive immune systems usually becomes apparent when affected animals show unusual susceptibility to infectious or parasitic diseases.

Con't..

- These diseases may be due to pathogenic organisms or, if the defect is very severe, opportunistic infections by organisms that are not normally able to cause disease.
- A condition resulting from a genetic or developmental defect in the immune system is called a primary immunodeficiency. In such a condition, the defect is present at birth although it may not manifest itself until later in life.

- Secondary immunodeficiency, or acquired immunodeficiency, is the loss of immune function and results from exposure to various agents. By far the most common secondary immunodeficiency is acquired immunodeficiency syndrome, or AIDS, which results from infection with the human immunodeficiency virus 1 (HIV-1).

Immunodiagnostic techniques

By Betelihem Tegege (DVM, MVSc in vet. Microbiology)

Immunodiagnostic techniques

- Immune responses are used in two ways to diagnose disease.
- First, specific antibodies may be used to detect or identify an antigen of interest. These antigens can be associated with an infectious agent, or can simply be molecules that need to be located or measured.
- Second, by detecting specific antibodies in serum, it is possible to determine whether an animal has been previously exposed to an infectious agent.

- This may establish a diagnosis or determine the degree of exposure of the population to that agent.
- The measurement of antigen-antibody interactions for diagnostic purposes is called serology.

- ❖ Serological techniques can be classified into three broad categories.
- Primary binding tests directly measure the binding of antigen to antibody.
- Secondary binding tests measure the results of antigen-antibody interaction in vitro.
- The third category, in vivo tests, measure the actual protective effect of antibodies in an animal

I. Primary binding tests

- Performed by allowing antigen (Ag) and antibody (Ab) to combine and then measuring the immune complexes formed
- In order to measure these reactions, one of the reactants must be chemically labeled
 - ✓ Radioisotopes (Tritium [H^3], carbon 14, iodine 125)

- ✓ Fluorescent dyes (Fluorescein isothiocyanate [FITC], Rhodamine)
- ✓ Enzymes (Horseradish peroxidase [HRP], alkaline phosphatase)

Examples of primary binding tests:

- ☐ Radioimmunoassays
- ☐ Immunofluorescence Assays
- ☐ Enzyme-Linked Immunosorbent Assays (ELISA)
- ☐ Western Blotting

Immunofluorescence Assays

1. Fluorescent antibody tests (FAT)

- Widely used in the serological diagnosis of bacterial, viral, fungal, and parasitic diseases

Advantage

- Sensitive and give reproducible results.
- **Sensitivity** is the ability of a test to correctly classify an animal as diseased (detect the positive results)

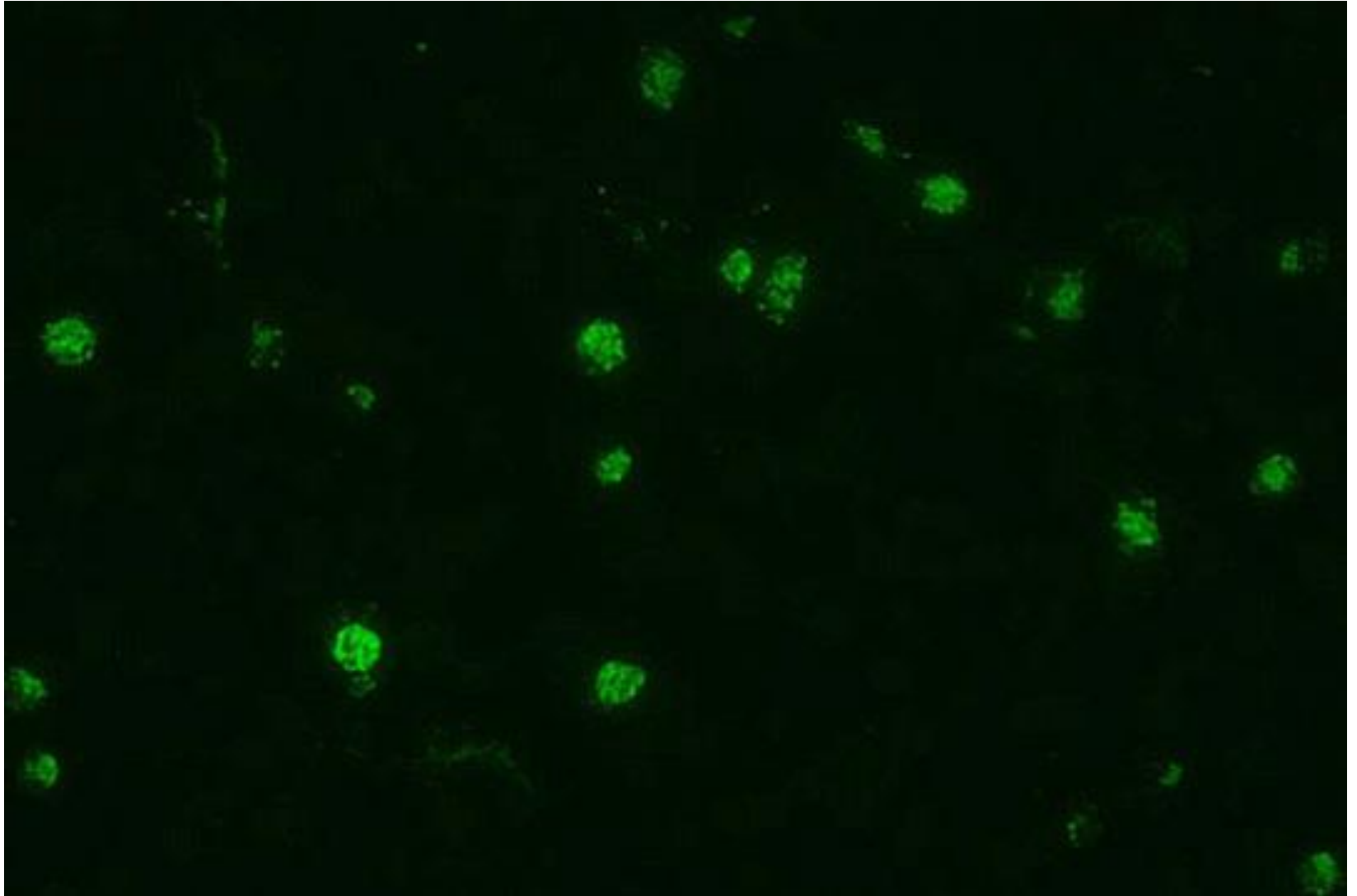
Disadvantages

- Special training is needed to perform and read Immunofluorescence tests.
- Fluorescence microscope and high quality reagents are required.

Principle

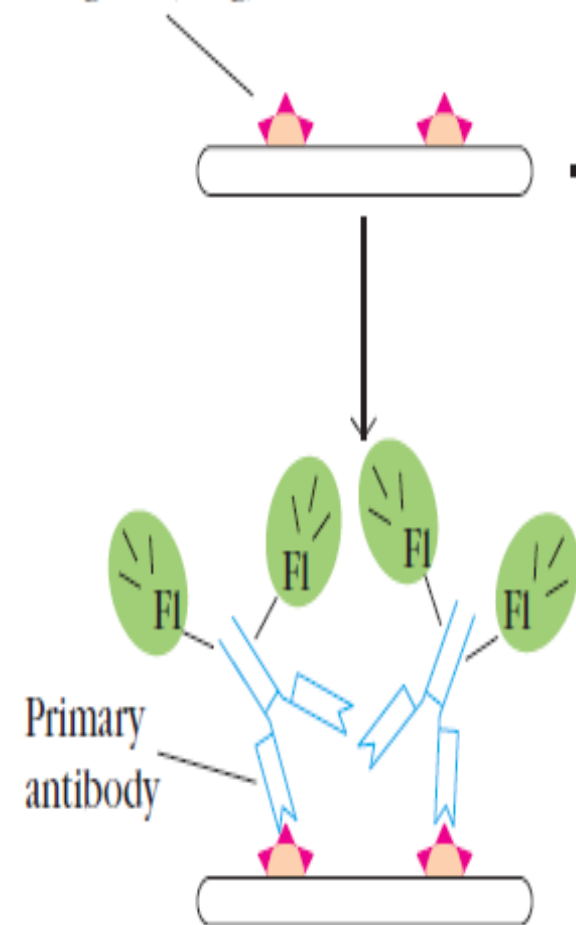
- Fluorescent dyes (fluorochromes) illuminated by UV lights are used to show the specific combination of an **antigen** with its **antibody**.
 - The **antigen-antibody complexes** are seen fluorescing against a dark background.
- **Fluorescein isothiocyanate** [FITC] emits visible green light
- **Rhodamine** emits red light

Immunofluorescence Assays

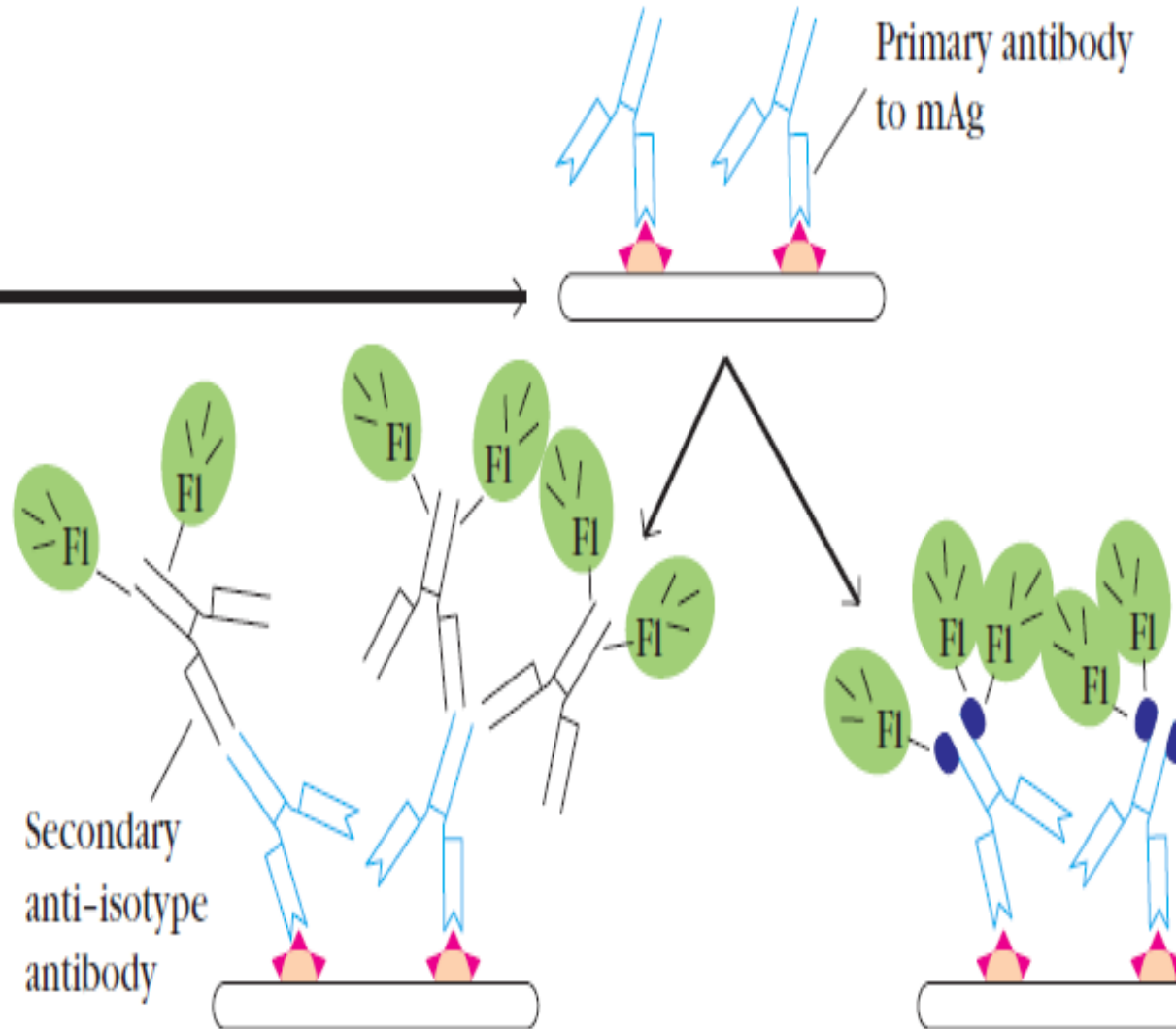


- **Direct FAT** is used to detect and identify an **unknown antigen** in specimens
- The **indirect FAT** is used in two main ways:
 - ✓ To detect and identify **unknown antigen** in specimens
 - ✓ To detect **antibodies** in a patient serum using a **known antigen** (microorganism).

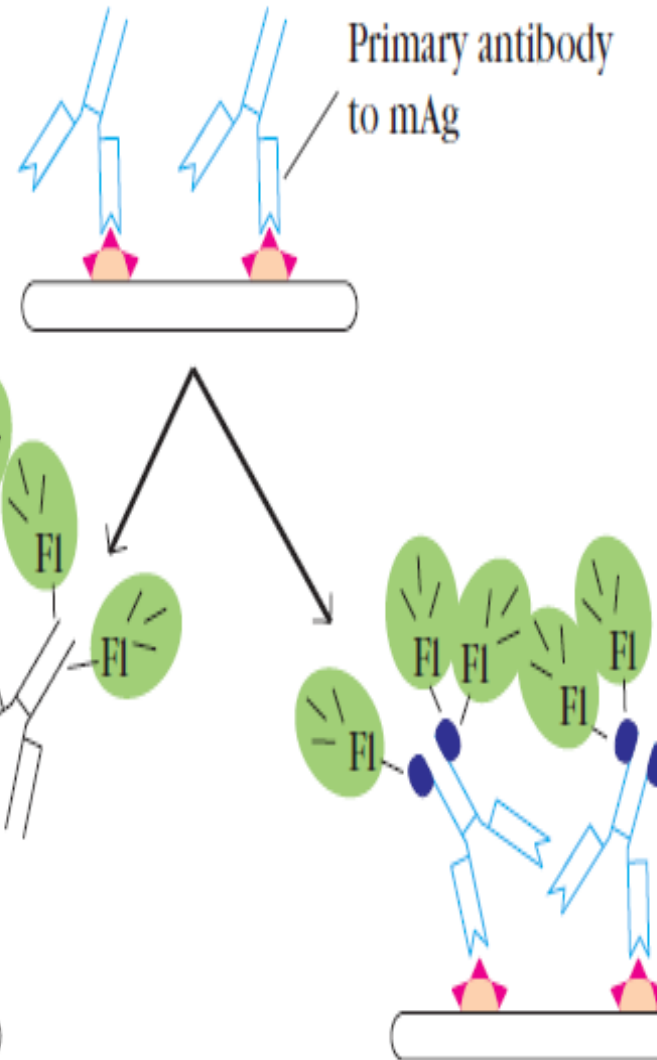
Cells with membrane antigens (mAg)



(a) Direct method with fluorochrome-labeled antibody to mAg



(b) Indirect method with fluorochrome-labeled anti-isotype antibody



(c) Indirect method with labeled protein A

Enzyme-Linked Immunosorbent Assays (ELISA)

- ELISA techniques are becoming increasingly used in the diagnosis of microbial infections.
- They are **specific**, **sensitive**, and require only a small amount of specimen.
- **Sensitivity** is the ability of a test to correctly classify an animal as diseased
- **Specificity** is the ability of a test to correctly classify an animal as healthy.
- Large numbers of specimens can be tested at one time

Principles

- As its name suggests, the **enzyme linked-immunosorbent assay** uses an **enzyme system** to show the specific combination of an **antigen** with its **antibody**.
- An **enzyme conjugated** with an **antibody** reacts with a **colorless substrate** to generate a **colored** reaction product.
- A number of variations of ELISA have been developed

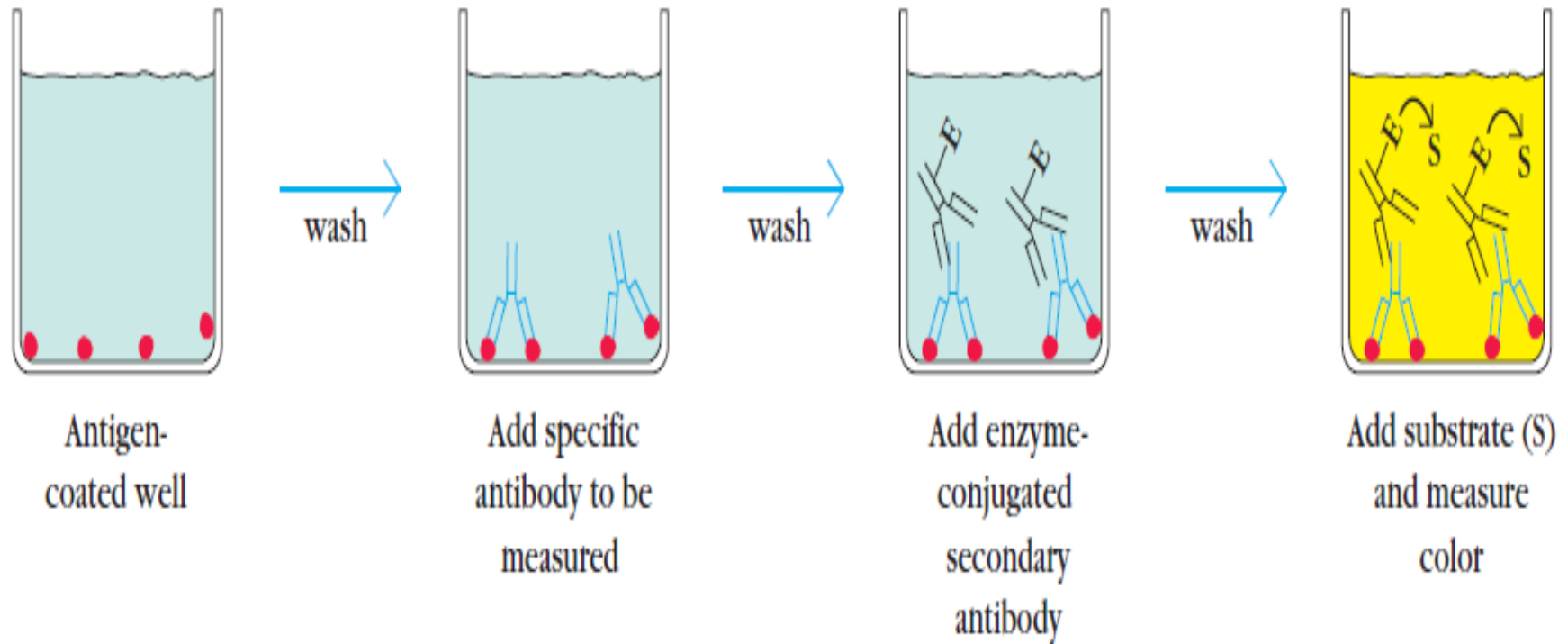
Indirect ELISA

- The most common form of ELISA is used to detect and measure specific antibodies.
- microwells in polystyrene plates are first filled with an antigen solution
- The serum under test is added to the wells. Any antibodies in the serum will bind to the antigen layer.

- After incubation and washing to remove unbound antibody, the presence of any bound antibodies can be detected by adding a solution containing an antiglobulin chemically linked to an enzyme.
- This labeled antiglobulin binds to the antibody and, following incubation and washing, can be detected and measured by adding a solution containing the enzyme substrate.

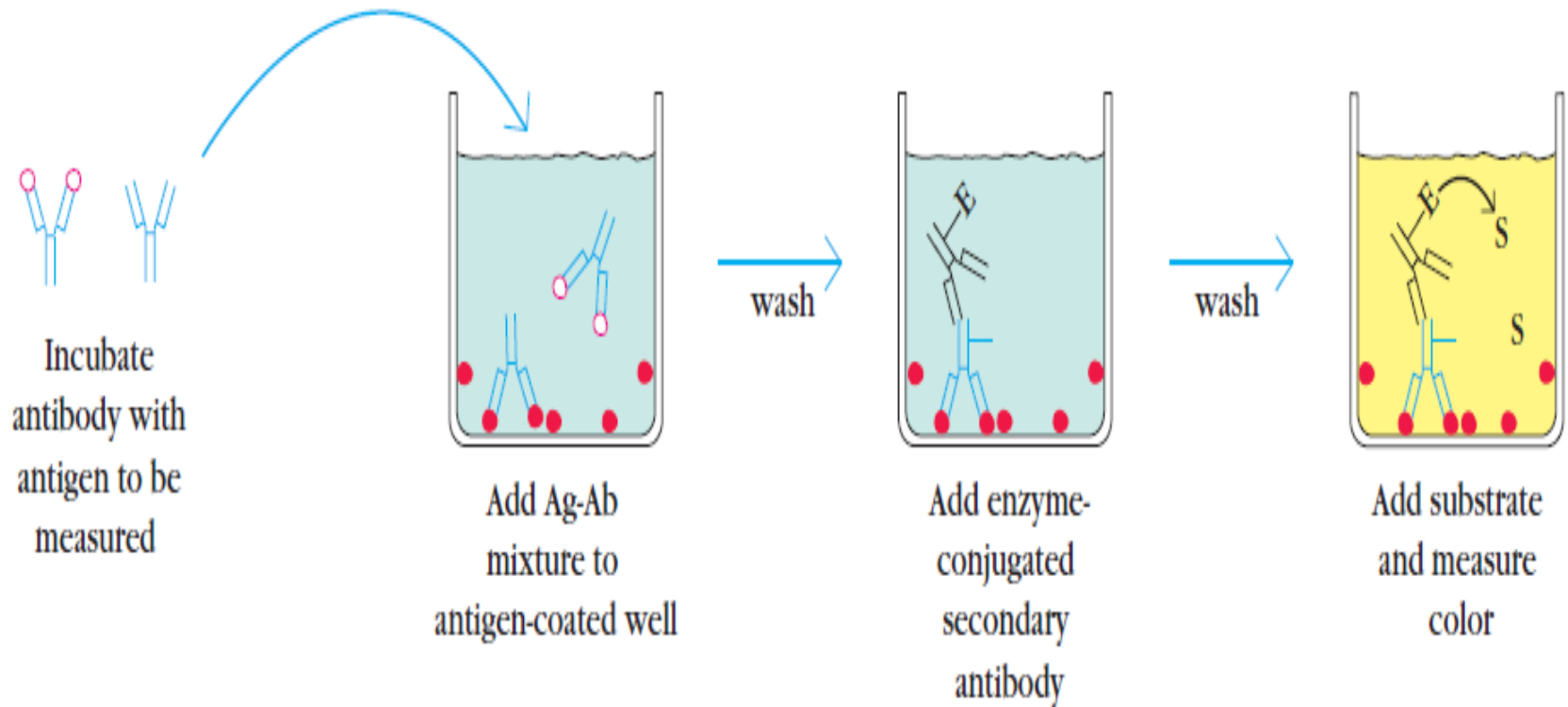
- The enzyme and substrate have been selected to ensure that a colored product develops in the tube.
- The intensity of the color that develops is therefore proportional to the amount of enzyme-linked antiglobulin that is bound, which in turn is proportional to the amount of antibody present in the serum under test.
- The color intensity may be estimated visually or, preferably, by spectrophotometry

Indirect ELISA

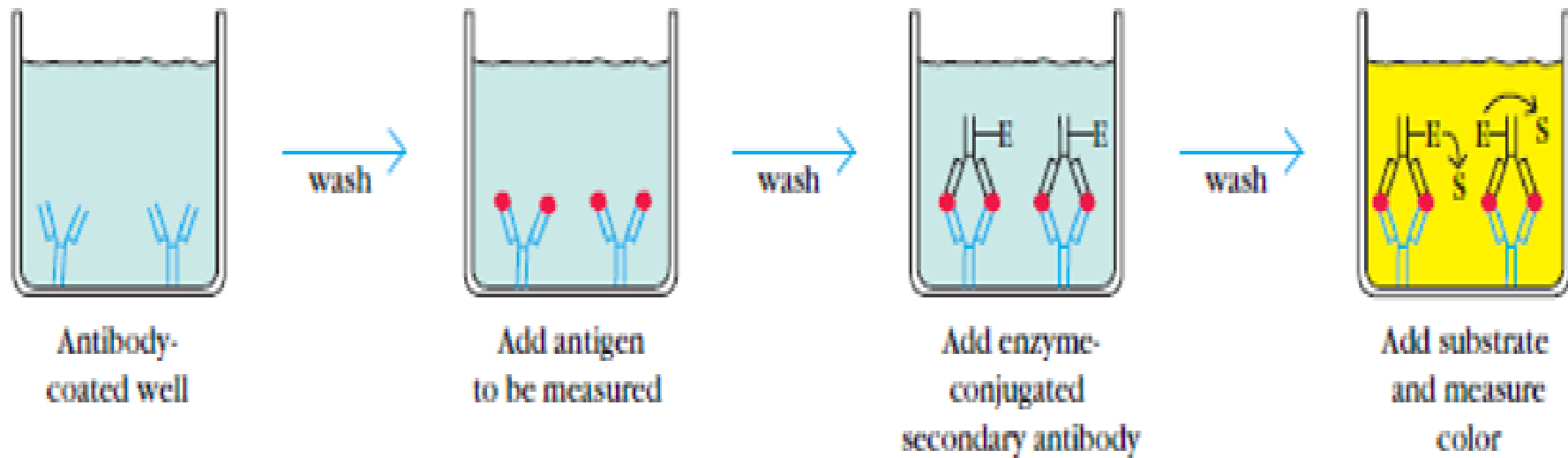


The indirect ELISA is used in the diagnosis of several parasitic infections and is being increasingly used in the diagnosis of bacterial, fungal and viral infections.

Competitive ELISA



- In the competitive assay, the higher the concentration of antigen in the original sample, the lower the absorbance. It can be used to measure viral antigens



- Because this test involves the formation of antibody-antigen-antibody layers, it is called **sandwich ELISA**.
- It is used to detect circulating virus in blood from cats with feline leukemia.

Radioimmunoassays

- The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody.

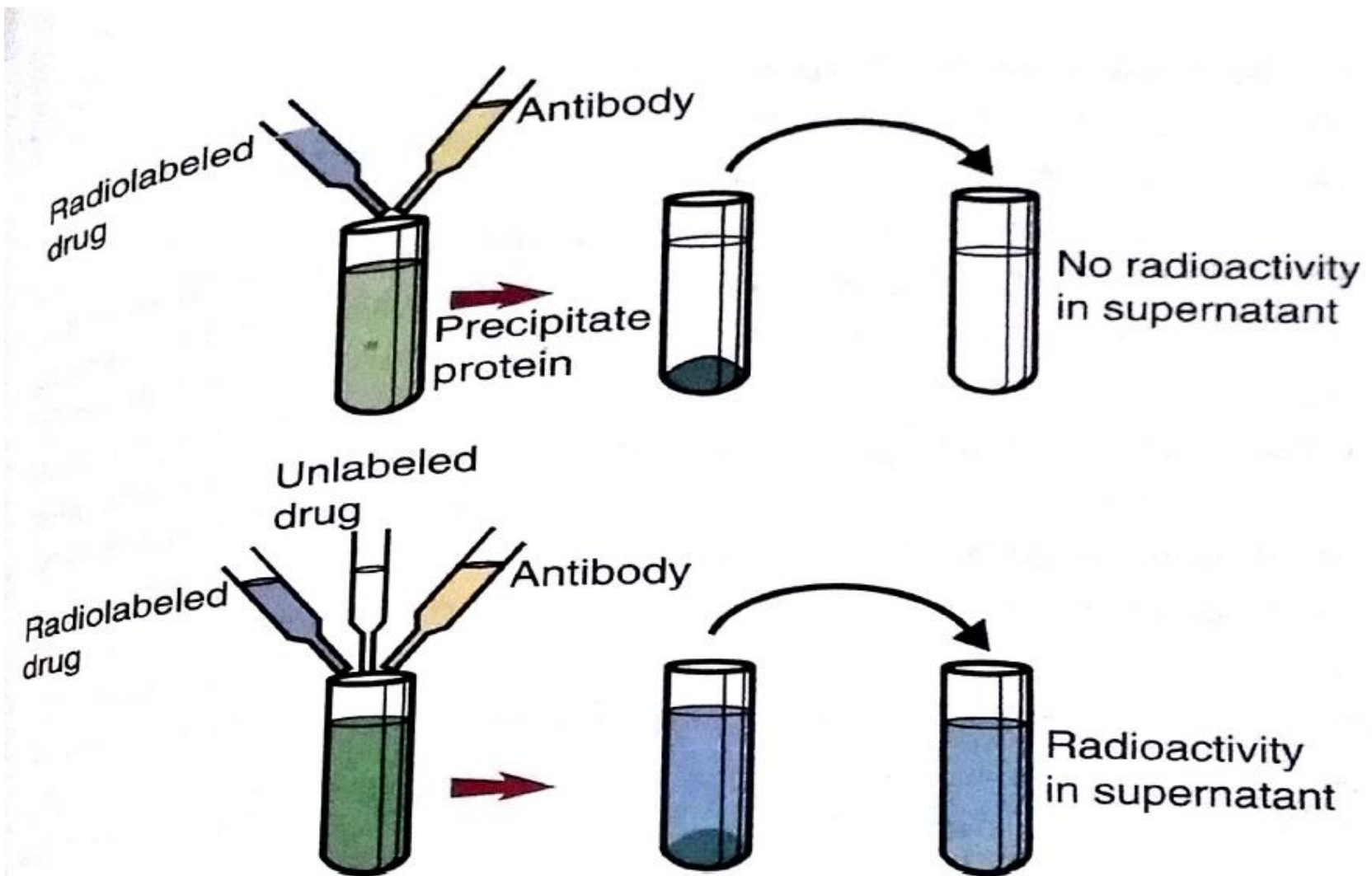
Advantage

- Very sensitive ($0.00005\mu\text{g/mL}$)

Disadvantage

- Expensive and Hazards of radioactivity and the need to safely disposal of radioactive material

- When radio labeled antigen is mixed with its specific antibody, the two combine to form immune complexes that can be precipitated out of solution. Any **radioactivity** remaining in the supernatant fluid is due to the presence of **unbound antigen**.
- If **unlabeled antigen** is added to the mixture, before adding the antibody, it will **compete** with the **radioactive antigen** for antibody-binding sites. As a result, some labeled antigen will be unable to bind, and the amount of radioactivity in the supernatant will increase.



Pictorial presentation of Radioimmunoassay

II. Secondary binding tests

- The reactions between **Ags** and **Abs** are commonly followed by a **secondary reaction**.
- If **Abs** combine with soluble **Ags** in solutions, the resulting complexes may **Precipitate**
 - **Abs** that aggregate soluble **Ags** are called **Precipitins**

- **Abs** binding to particulate Ags (e.g. bacteria or RBCs), may make them **clump** or **agglutinate**
 - **Abs** that produce **agglutination** reactions are called **agglutinins**
- Neutralization of bacteria, viruses, or toxins; and
- Activation of the complement system.

Agglutination Tests

Principle of agglutination tests

- Agglutination is the visible clumping together of bacteria, cells, or particles, by an antigen combining with its specific antibody. The resulting clumps are referred to as agglutinates.
- Agglutination tests can be performed:
 - a) On slides
 - b) In tubes
 - b) In microtitration plates

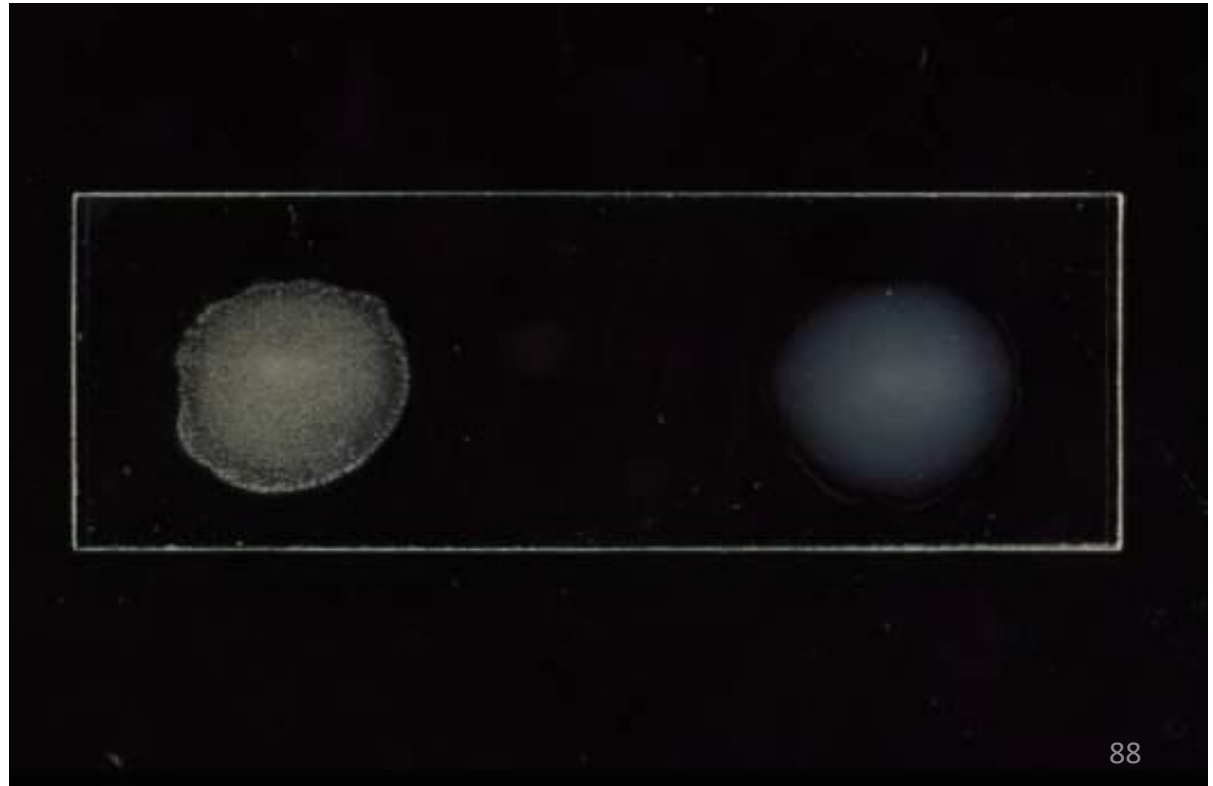
A) Slide agglutination tests

- These are rapid, easily performed techniques that give a reaction in minutes or even seconds. Example: Rose Bengal Plate Test (RBPT)
- **Principle:** RBPT is a rapid plate agglutination test for the detection of agglutinating antibodies by using inactivated *Brucella* cells, stained with Rose Bengal and suspended in an acid buffer.

- The acid pH of the suspension prevents the non-specific agglutination of the bacteria, increasing the specificity of the test. Rose Bengal is used to mark the antigen and make its clumping visible. agglutinating IgM, IgG and IgA antibodies contribute to the reactions.

Test Interpretation

- Any degree of agglutination = presence of antibodies directed against *Brucella* organism
- No agglutination = absence of antibodies directed against *Brucella* organism



B) Tube agglutination tests

- In tube tests, agglutination occurs in a larger volume of fluid
- Tube tests are usually more sensitive than slide tests.
In this tube agglutination test, serum is diluted serially and then antibody level is measured by adding standard antigenic suspension.

C) Microtitration agglutination tests

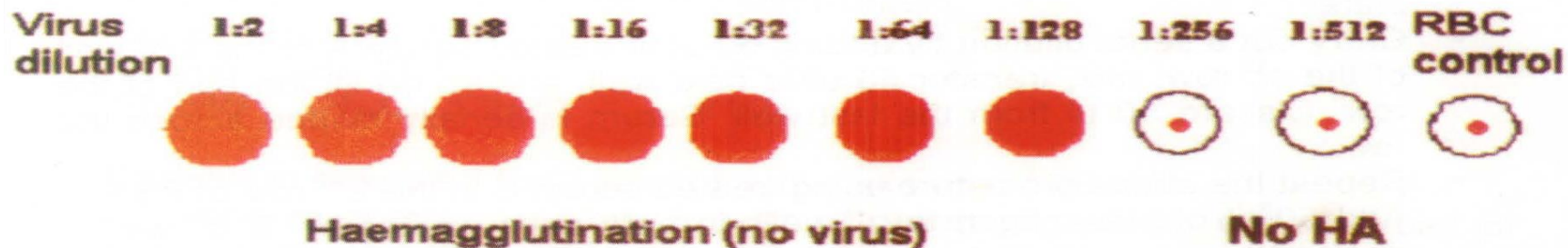
- These techniques are performed in microtitration plates. They are more sensitive, more economical, easier to perform, and usually give quicker results

Example: Haemagglutination inhibition test (HI)

- This technique is used to detect antibodies against Arboviruses and Influenza viruses. These viruses are able to agglutinate red cells because they possess haemagglutinins on their outer surfaces.

- In the haemagglutination inhibition (HI) test, the animal's serum is reacted with a suspension of known viral antigen. If the corresponding antibody is present, it will coat the haemagglutinins (antigens) on the viral particles and so prevent haemagglutination when red cells are added. The viral antigen should be titrated.

A)



Titre of virus haemagglutinin is 128

RBC precipitate

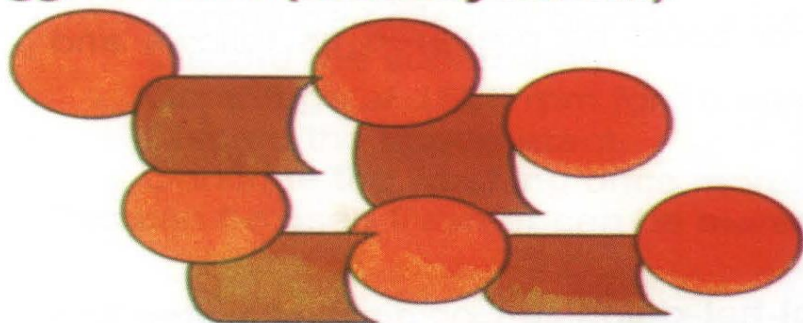
B)



No agglutination (antibody present)



Agglutination (antibody absent)



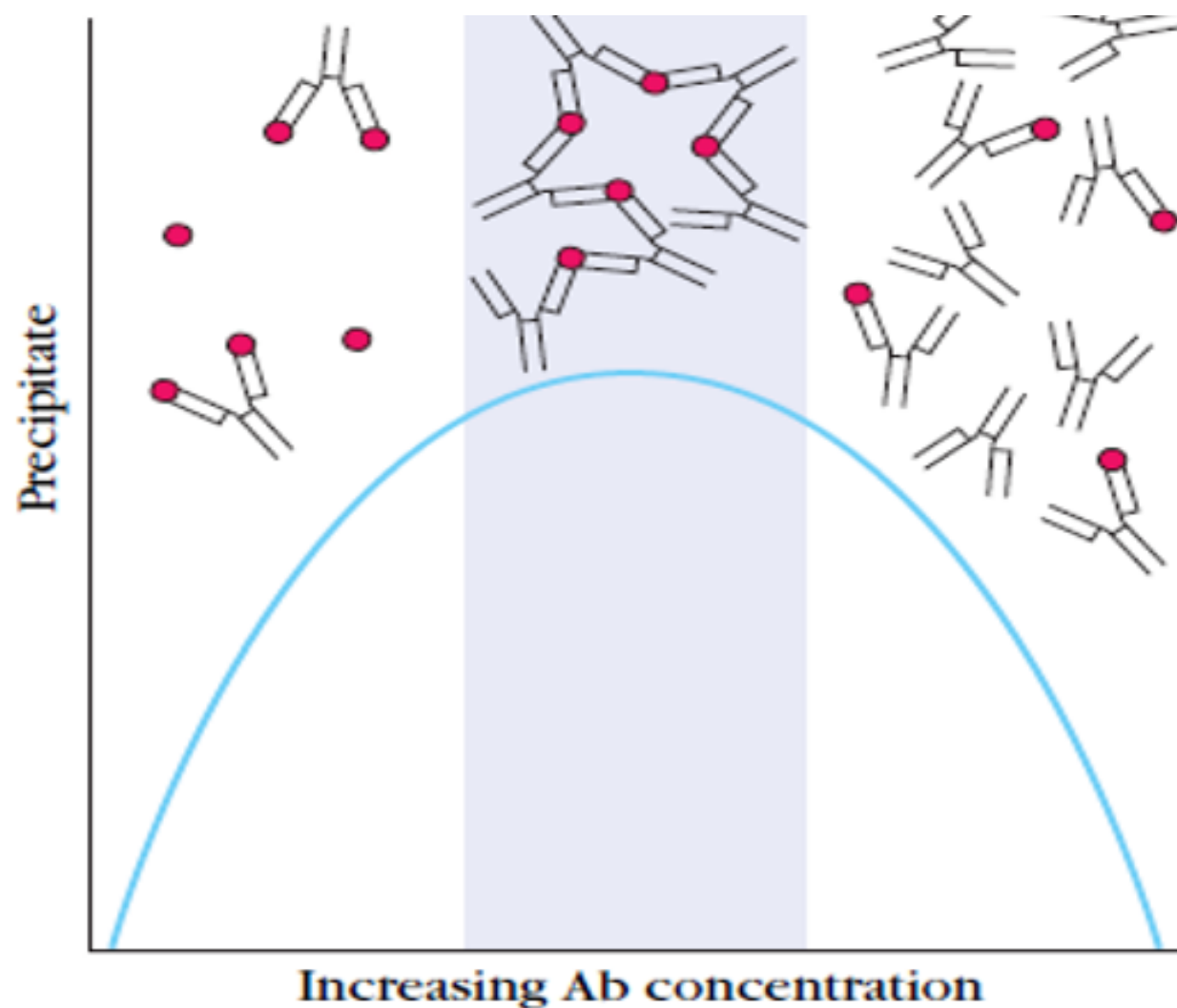
Precipitation Tests

- Precipitin techniques are used to detect and identify antigens in specimens, extracts, and cultures, and to detect and quantify antibodies in serum. Compared with agglutination tests, precipitin techniques require more experience in their performance and interpretation.

Principle

- In precipitation tests, the antigen and antibody are in a soluble form and combine to form a visible precipitate. Various systems are available in which precipitation tests are performed, either in semisolid media such as agarose, or cellulose acetate. Agarose is a transparent, colorless, neutral gel.

- Immunoprecipitation techniques rely on the ability of antibodies to bind to more than one antigenic determinant on a single antigen, thus forming a large complex that will fall out of solution.
- The test can be done in solution, or on solid agar/gel matrices. In solution, it forms precipitates.

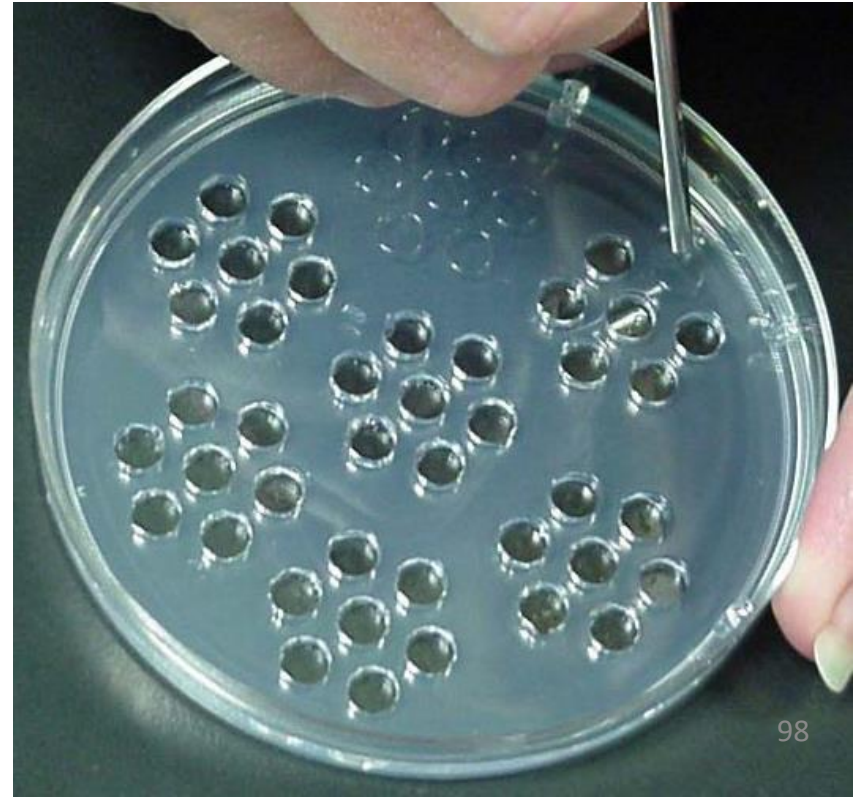
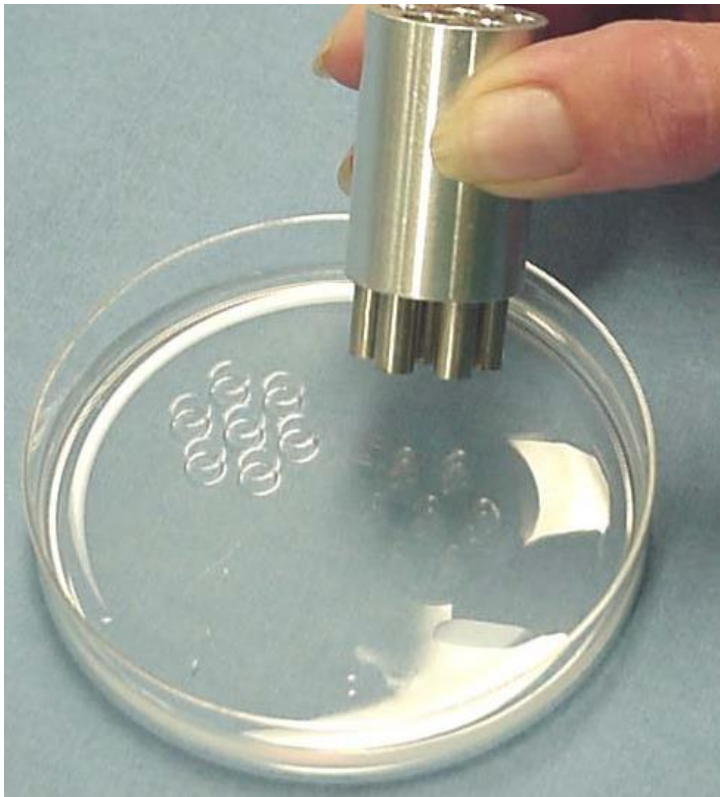


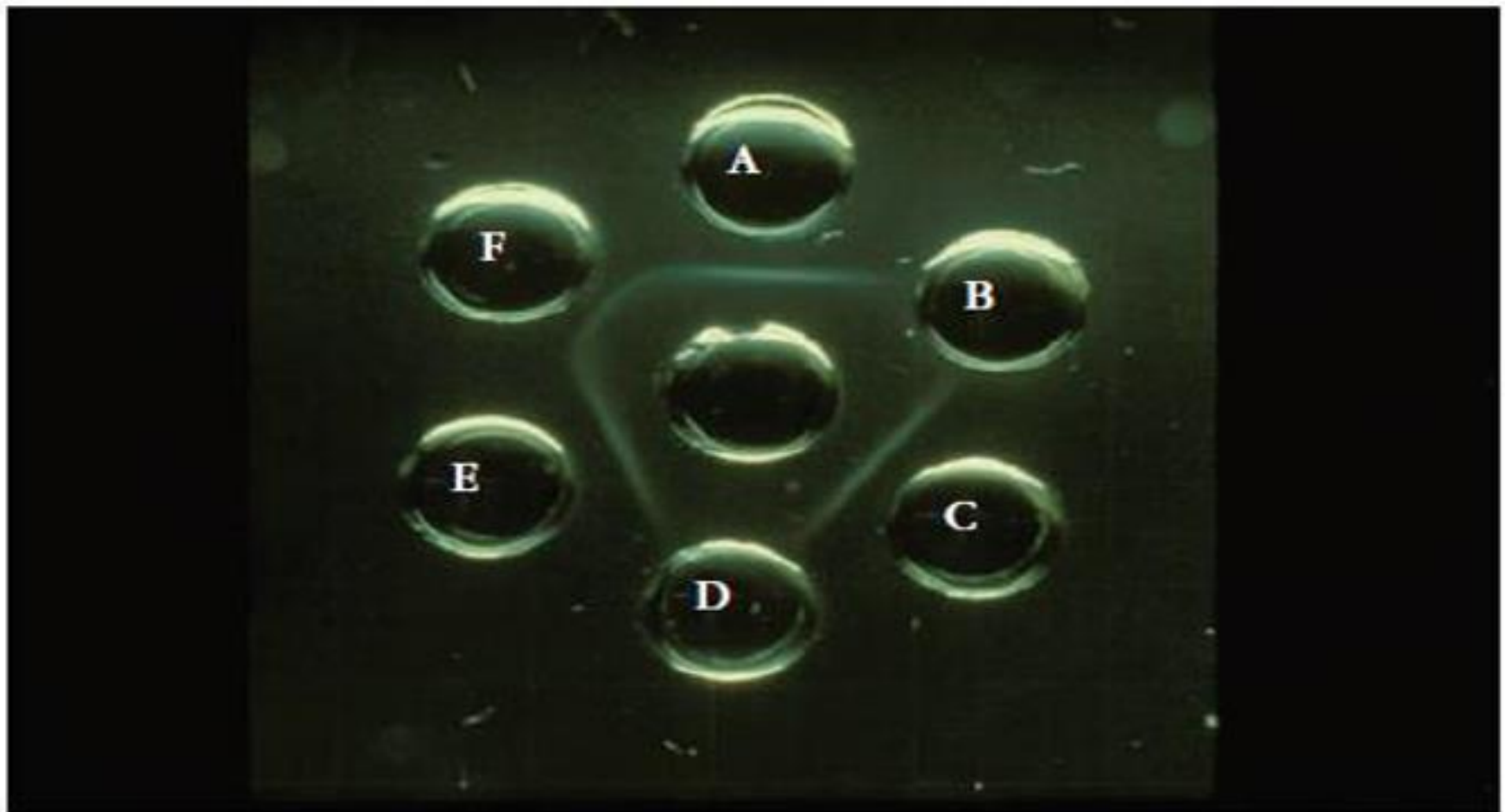
Immunoprecipitation in solution. When bi- or multivalent antibodies are mixed in solution with antigen, the antibodies can form cross-linkages with two or more antigen molecules, leading to the formation of a cross-linked precipitate (middle

- Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in a gel matrix, a visible line of precipitation (lattice) will form.
- As in a precipitation reaction in solution, visible precipitation occurs when the concentrations of antibody and antigen are equivalent to one another.
- Both antigen and antibody diffuse radially from wells toward each other.

E.g. Agar Gel Immunodiffusion (AGID) Test

- The diffusion of soluble antigens and/or antibodies toward each other leading to their precipitation in a gel matrix.

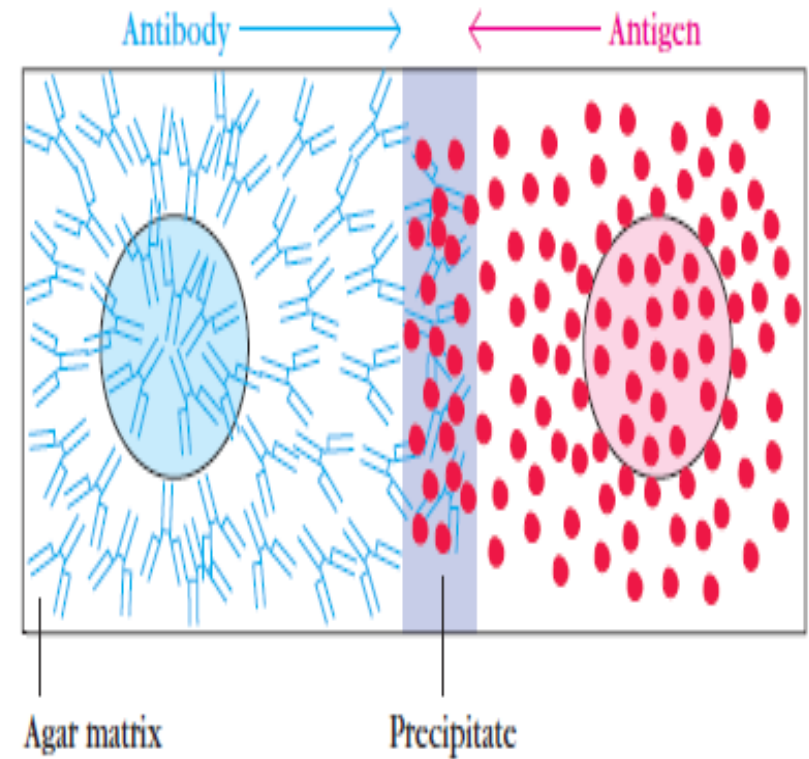




Immunodiffusion in agar gels can be used to assay for the presence of antibodies and determine cross-reactivity patterns between complex antigens and antibody samples. In this example, viral antigen was placed in the center well, and serum samples from different individuals were introduced into each of the surrounding wells. Note that the serum samples of individuals B and D are negative for antiviral antibodies, in that



DOUBLE IMMUNODIFFUSION



Complement Fixation Tests (CFT)

- CFT is best performed in reference laboratories where facilities exist for the careful standardization and control of reagents, which these tests require. Usually used as confirmatory test for bacteria like Brucella

Principle of CFT

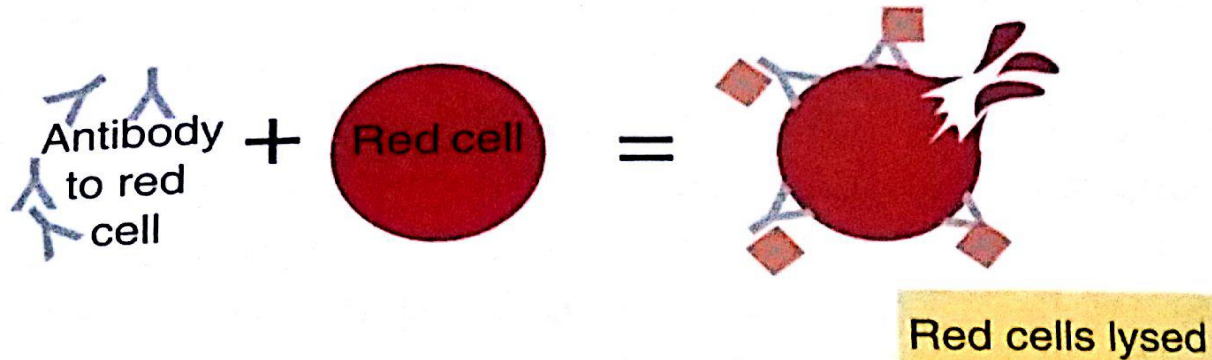
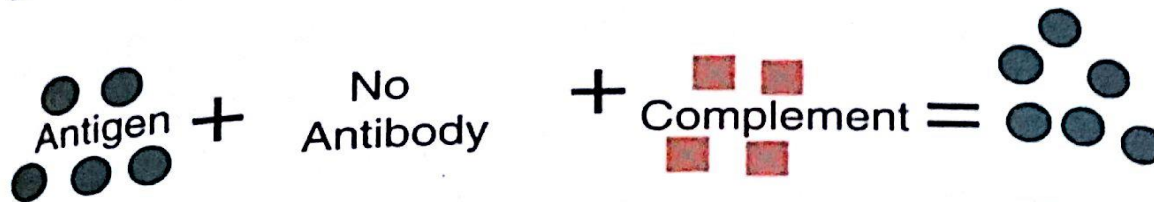
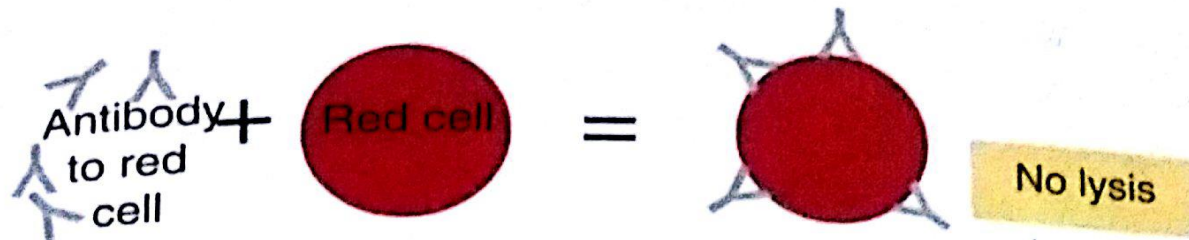
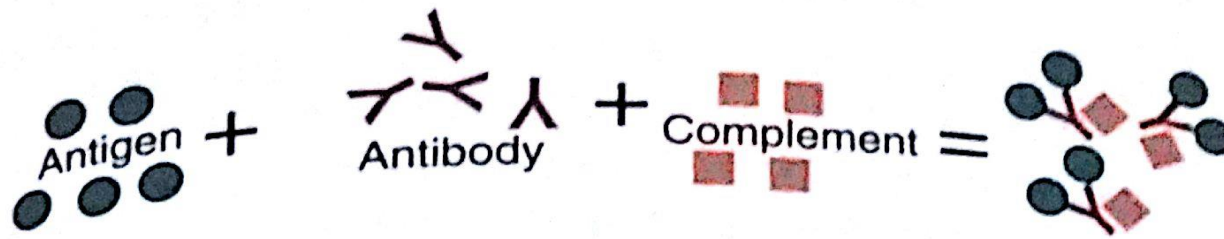
- It is used to detect and quantify antibody that does not agglutinate or precipitate when reacted with its antigen, but can be demonstrated by its use, or fixation, of complement.

The complement fixation test system consists of two components:

- The first component is an indicator system consisting of a combination of sheep red blood cells, anti sheep red cells antibody and exogenous source of complement, usually guinea pig serum. When these three components are combined in an optimum concentration, the anti sheep red blood cell antibody complexes and causes cell lysis.

- The second component consists of a known antigen and inactivated animal's serum (the serum under test deprived of its complement by heating at 56°C).
- Antigen-antibody reactions lead to immune complex formation that produces complement fixation via the classical pathway. When these complexes are on bacteria, red cells or other cells, the complement brings about the lysis of the cells involved.

Complement fixation test



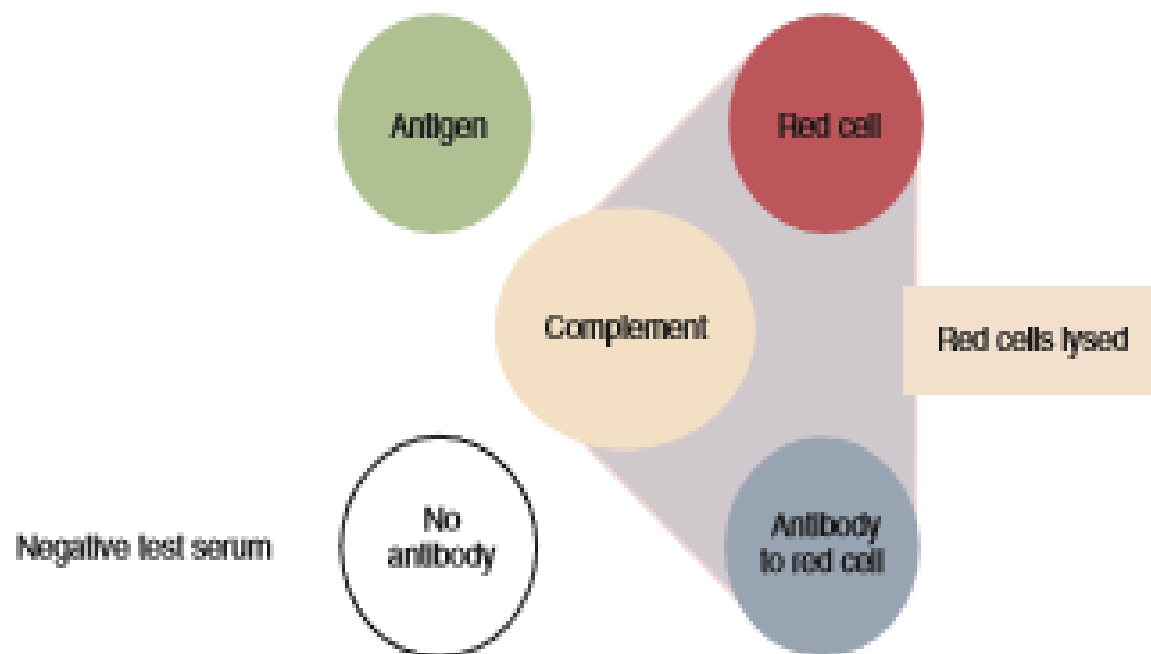
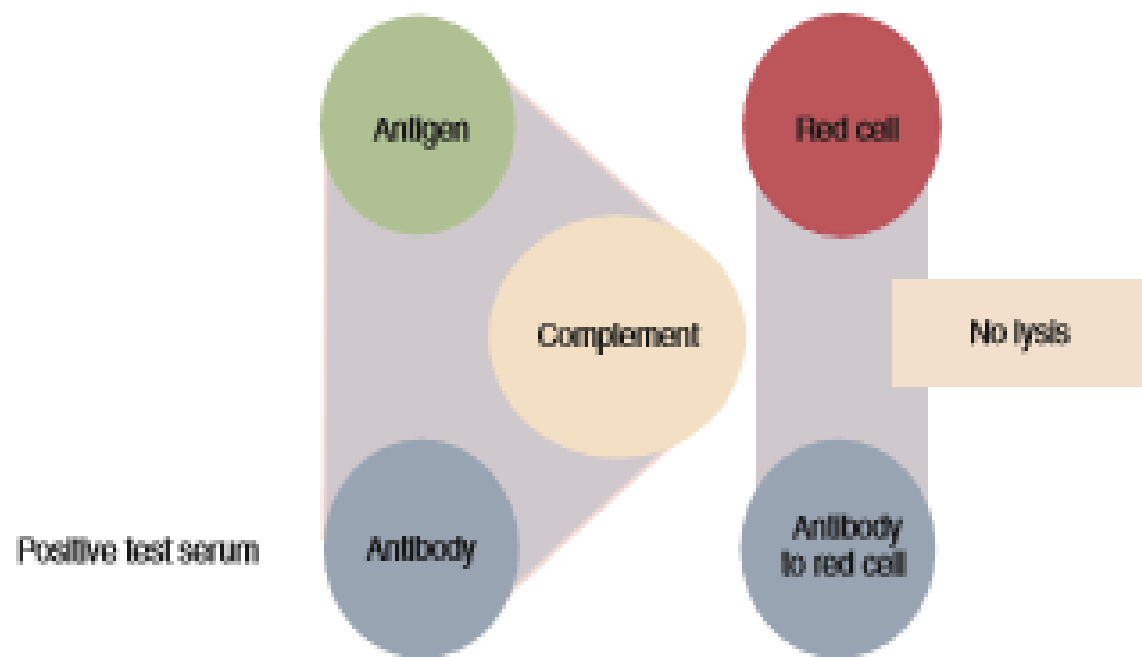


FIGURE 41-30 The principle of the complement fixation test. Complement, if fixed by antigen and antibody, is unavailable to lyse the target cells in the indicator system. In the absence of antibody the complement remains unfixed and is available to lyse the indicator system.

(Modified from Roitt, *Essential Immunology*. Blackwell Science, 1971, Oxford.)

Interpretation:

- Lysis of the indicator sheep cells indicates both a lack of antibody and negative complement fixation test.
- If the sick animal's serum does contain a complement-fixing antibody, a positive result will be demonstrated by the lack of hemolysis

VACCINES

- **Immunization:** The process of producing a state of immunity in a subject

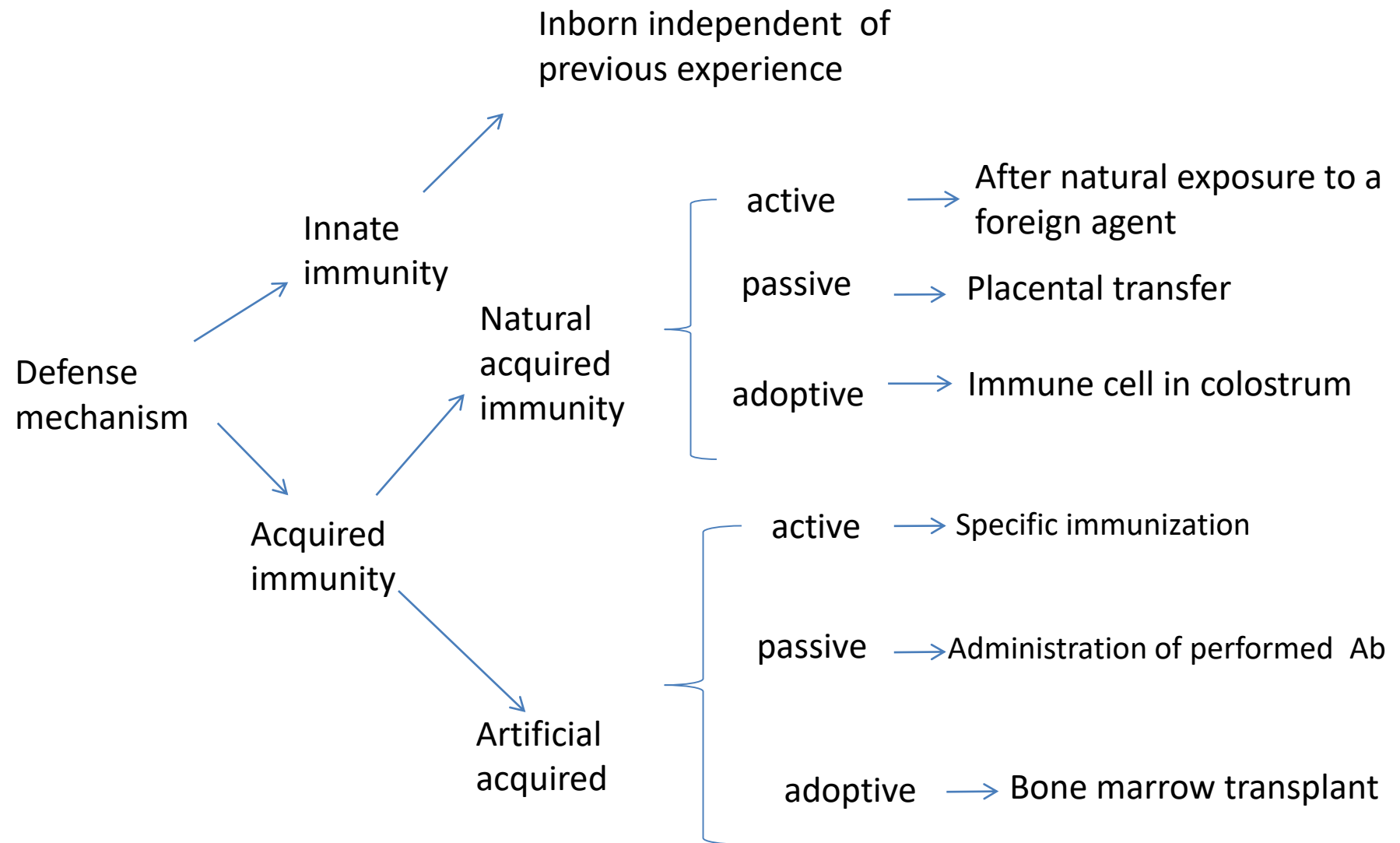
Types of immunization procedures

- There are two basic methods by which any animal may be made immune to an infectious disease:
 - a) **Passive immunization**
 - b) **Active immunization**

A) Passive immunization

Achieved by:

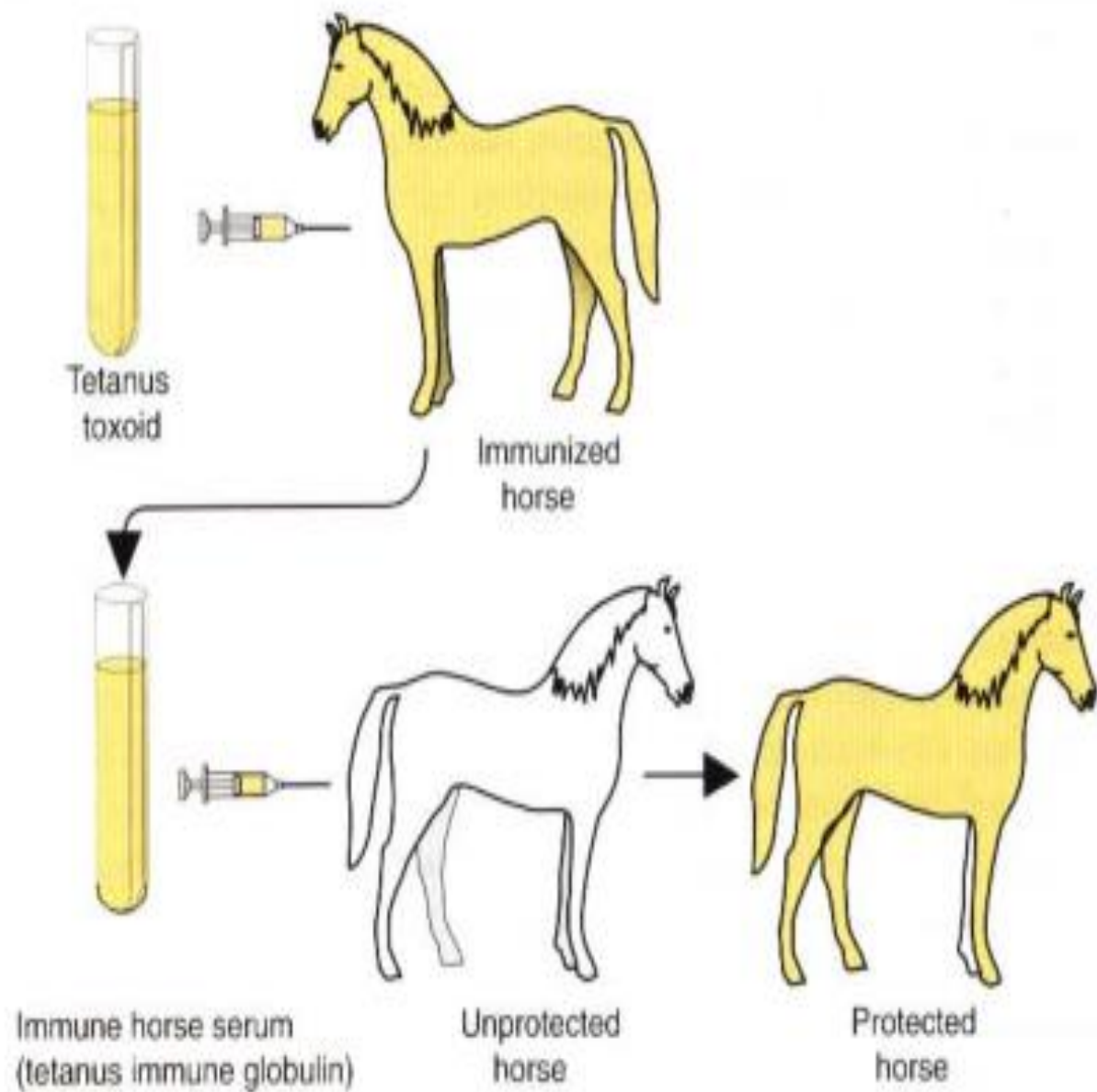
- Transfer of preformed antibodies from dam to the developing fetus across placenta
- Transfer of maternal antibodies to newborns through colostrum



→ Injecting a recipient animal with preformed antibodies (immunoglobulins) in donor animals by active immunization

e.g. anthrax (cattle), distemper (dog), panleukopenia (cat),
Tetanus (horses)

Figure 1-6. Transfer of immunity to tetanus by means of serum derived from an immunized horse. This clearly demonstrates that antibodies in serum are sufficient to confer immunity to tetanus.



Advantage of passive immunity

- Confer immediate protection

Disadvantage

- No memory response and the protection provided is short-lived
- Elicit an immune response when given to other species of animal (immunoglobulins, mAbs)
- Transmission of **infections** from donor to recipient

B) Active immunization

- Achieved by **natural infection** with a **microorganism**, or
- **Acquired artificially** by administration of a **vaccine**

Advantage

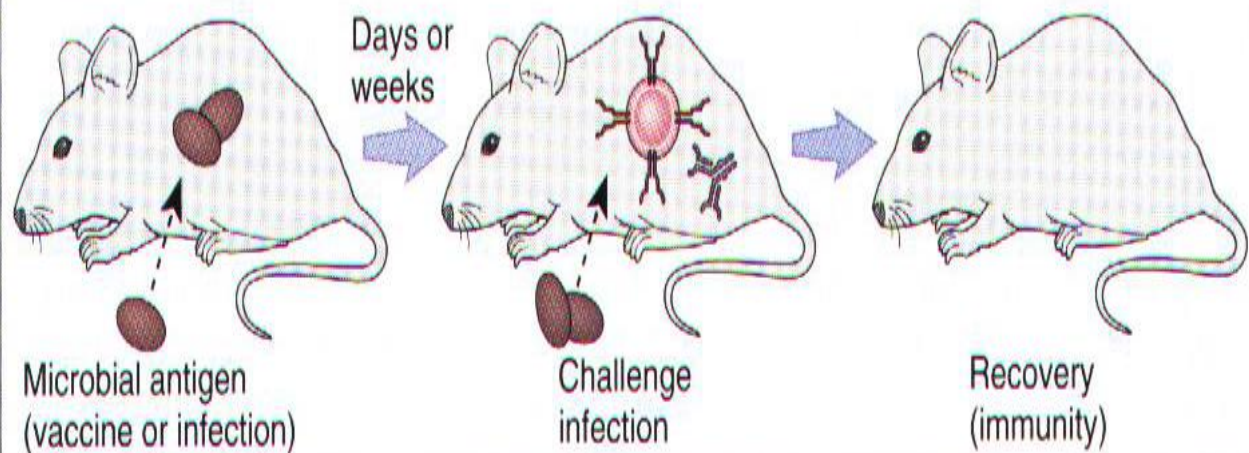
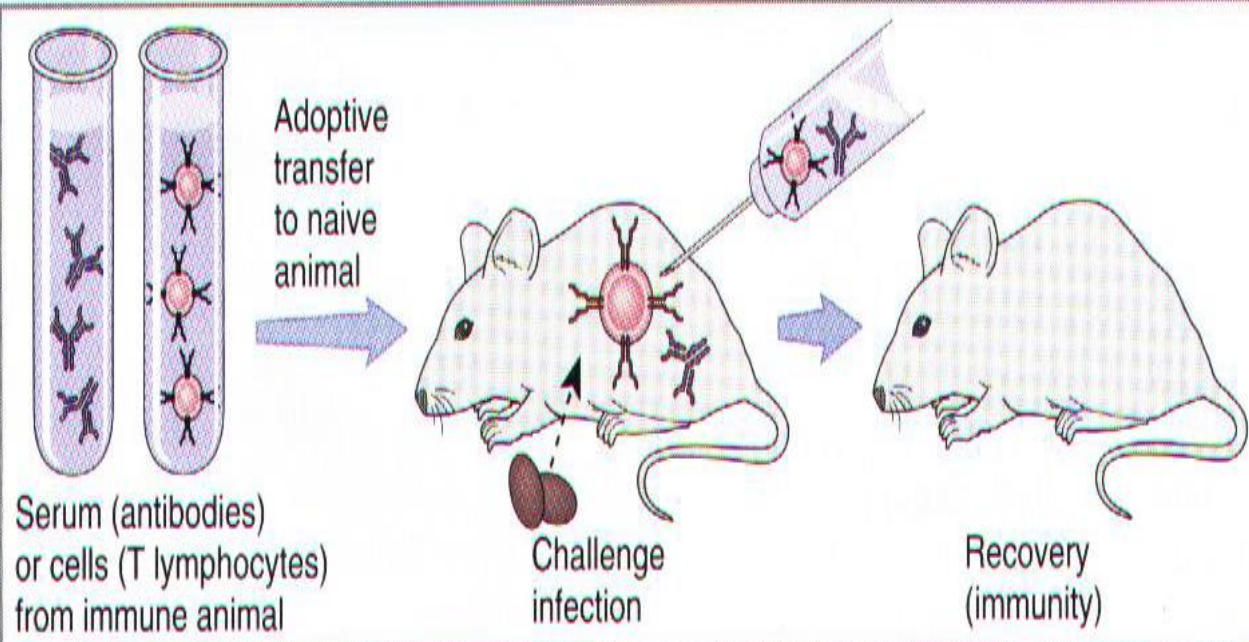
- Provide long-lasting immunity
- Capable of re-stimulation by repeated injections of antigen or exposure to infection

Disadvantage

- Protection is not conferred immediately

Passive Vs Active Immunization

Feature	Passive	Active
Protection	Immediate	Requires 7-10 days
Duration	Days to months	Years
Host immune system	Not involved	Required
Elements of response	Anti-pathogen Ab only	Anti-pathogen Ab, CTLs, Th cells
Memory	No	Yes
Uses	Immunocompromised individual unvaccinated individual where no vaccine exist	Immunocompetent individual individuals not naturally exposed to pathogen where a safe, efficacious vaccine exist

			Specificity	Memory
Active immunity	 Microbial antigen (vaccine or infection) Days or weeks Challenge infection Recovery (immunity)		Yes	Yes
	 Serum (antibodies) or cells (T lymphocytes) from immune animal Adoptive transfer to naive animal Challenge infection Recovery (immunity)		Yes	No

So what is vaccine?

Vaccine: A preparation of immunogenic material used to induce immunity against pathogenic organisms

Vaccination: Intentional administration of a harmless or less harmful form of a pathogen to induce a specific immune response that protects the individual against later exposure to the pathogen

Principle of vaccination

1. Primary immune response

- Response that occurs after the **first exposure** of an individual to a **foreign antigen**
- Characterized by relatively **slow kinetics** and **low magnitude** compared with the response after a **subsequent exposure** to the same antigen

2. Secondary immune response

- Response that occurs on **second exposure** to **previously** encountered antigen
- Characterized by **more rapid kinetics** and **greater in magnitude** than the **primary immune response**

- The principle of vaccination follows the above types of immune response:
 - That is, **initial administration** of the vaccine against specific pathogen will initiate **primary immune response**.
 - Then, **natural infection** by the **same pathogen** will induce **secondary immune response**, with ultimate elimination of the pathogen.

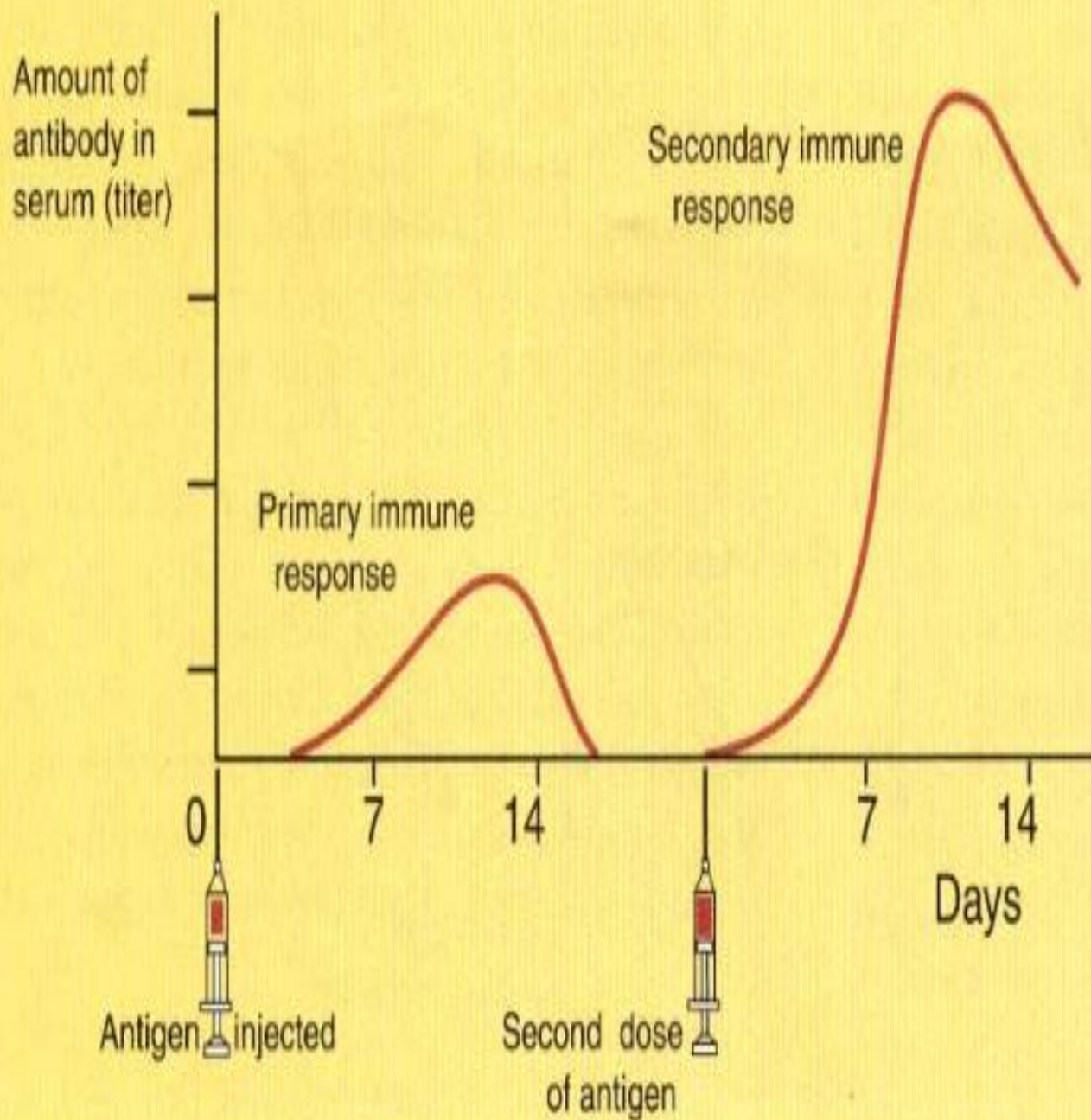
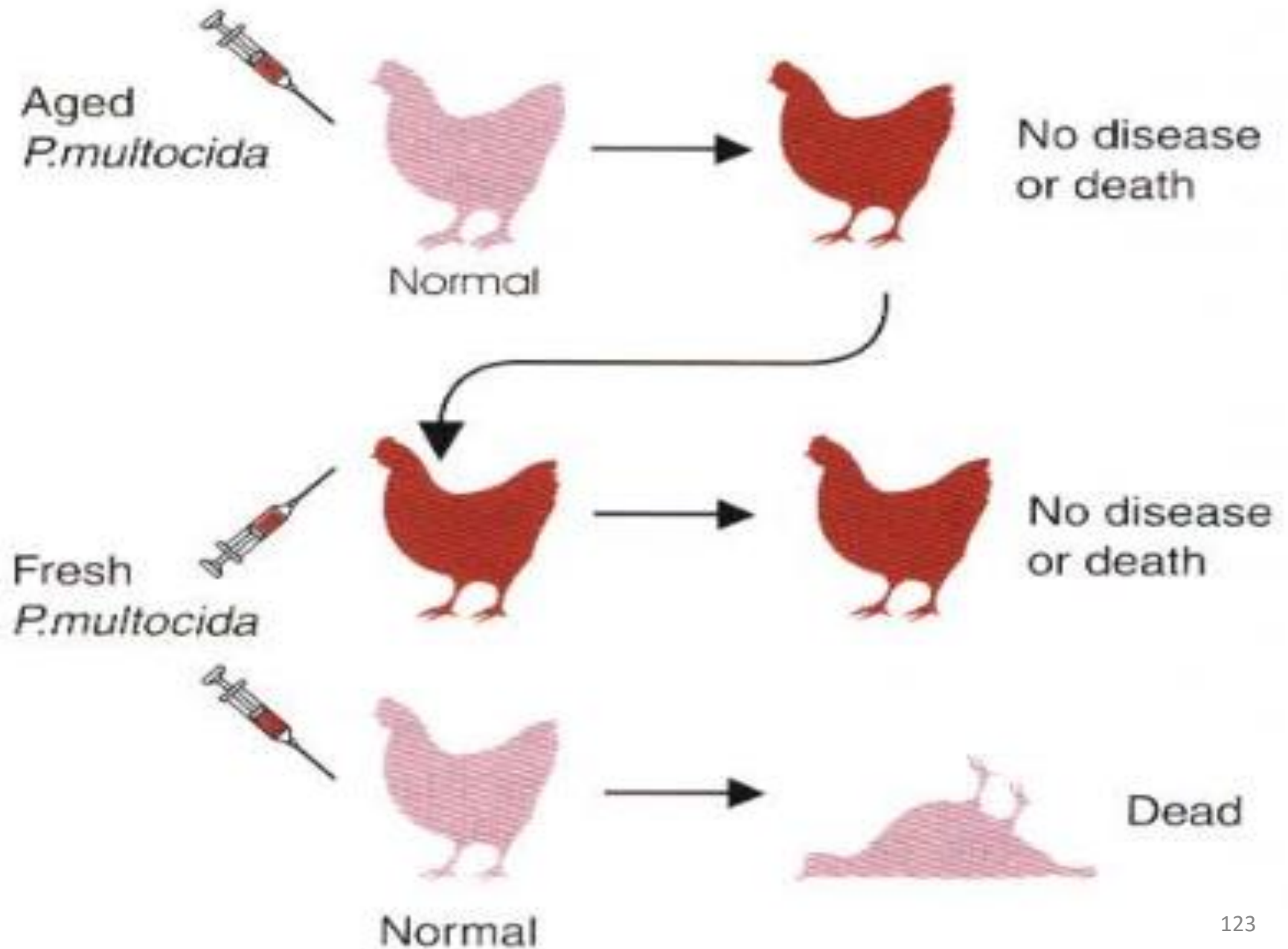


Figure 1-7. The characteristic time course of the immune response to an antigen as measured by serum antibody levels. Note the key differences between a primary and a secondary immune response.

Characteristics of ideal /perfect/ vaccine:

- It should give prolonged **strong immunity**.
- The immunity should be conferred on both the **animal immunized** and any **fetus** carried by it
- It should induce both **humoral** and **cellular** immunity

- It should be free of **adverse side effects**
- It should be cheap, stable, and adaptable to mass vaccination
- It should stimulate an **immune response** distinguishable from that resulting from **natural infection**



Routes of vaccine administration

- Most vaccines are administered by injection
- **Precaution while injection**
 - ✓ All needles must be clean and sharp
 - ✓ Skin at the injection site must be clean and dry
 - ✓ Vaccines are provided in a standard dose, and this dose should not be divided to account for body weight or age

A. Subcutaneous or intramuscular routes

- The simplest and most common method of vaccine administration
- Excellent for small numbers of animals and for disease in which systemic immunity is important
e.g. Rabies vaccine, Rinderpest, Anthrax

B) Intranasal route

- Induce both systemic and mucosal immune responses
 - Obviate/ avoid/the need for sterile syringes and needles
- e.g. *Streptococcus equi* infection in horses,
- Infectious bronchitis and Newcastle disease in poultry

C) Aerosolization

- Enables the vaccines to be inhaled by all the animals in a group

e.g. **Canine distemper** and **Newcastle disease**

D) Oral route

- Putting vaccines in feed and drinking water
 - Induce both **systemic** and **mucosal immune** responses
 - Avoid the need for sterile syringes and needles
- e.g. Newcastle disease and infectious laryngotracheitis vaccines in poultry

E) Intradermal and epidermal routes

- Avoid needle dangers and drawbacks
- Dose-sparing ability (smaller quantities of vaccine antigen could be delivered)

e.g. BCG (Bacille Calmette-Guerin), smallpox

F) Immersion

- Achieved by immersing fish in a dilute antigen solution
- Immersion results in the antigen being deposited on mucosal surfaces such as gills or oral cavity and some may be swallowed

First generation vaccines

- Consist of many of the common vaccines currently in use
- Its formulation involves
 - Cultivation and harvesting of organism,
 - purification and conversion either as suspension form or to freeze dried products

Con't..

- Originate from viruses or bacteria and can be divided into

A. Killed vaccines

B. Live attenuated vaccines

A) Killed vaccines

- Composed of killed or inactivated normal (wild type) infectious, pathogenic microorganisms
- **Methods of inactivation:**
 - ✓ Heat treatment
 - ✓ Gamma irradiation

Chemical treatment

- ✓ Formaldehyde
- ✓ Acetone
- ✓ Alcohol

Alkylating agents

- Ethylene Oxide
- Ethyleneimine
- Acetyleneimine and β -propiolactone)

Advantages

- Unlikely to cause disease through residual virulence
- No risk of reversion to wild type pathogen
- Lower development cost
- Safe to use and can be given to immunodeficient and pregnant individuals
- Stable on storage
- Unlikely to contain live contaminating organisms

- **Disadvantages:**

- Require use of adjuvant
- Since the microorganisms cannot multiply, a larger quantities are required to stimulate immunity

- Higher risk of producing hypersensitivity reaction
- Require repeated boosters to maintain the immune status of the host
- Induce a predominantly humoral antibody response
- Produce relatively short-lived immunity

Examples:

- ✓ Rabies vaccine
- ✓ FMD vaccine
- ✓ Equine herpesvirus-4 vaccine

Vaccines containing **killed bacteria** are called **bacterins**

- B. abortus* strain 45/20 vaccine
- E. coli* bacterins
- Mannheimia* bacterins, etc

B) Live attenuated vaccines

- Composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease
- The process of reducing virulence is called attenuation
- Under-attenuation will result in residual virulence and disease
- Over attenuation will result in an ineffective vaccine

Methods of attenuation

I. Adapting organisms to growth in unusual condition

E.g.1. **BCG strain** of *M. bovis* was rendered avirulent by being grown for **13 years** on **bile saturated medium**

2. *B.abortus* strain 19 was grown under conditions in which there was a **shortage of nutrient**
3. **Sterne Strain** *B. anthracis* was rendered **avirulent** by growing in **50% serum agar** under an atmosphere rich in **CO₂** so that it lost its ability to form a **capsule**

II. Growth in species to which they are not naturally adapted

E.g. 1. Rinderpest virus was attenuated by growing in **rabbit**

2. African horse sickness virus to **mice**

3. Canine distemper virus to **ferrets**

III. Growth in eggs

E.g. Flury strain of **rabies** was attenuated by prolonged passage in eggs

IV. Prolonged tissue culture

E.g. **Canine distemper** virus in **canine kidney** cells

Advantages

- Stimulate both humeral and cell-mediated responses
- Do not require use of adjuvant
- Confer long-lasting protection
- Some live vaccines can be given orally and induce mucosal immunity, which gives more protection at the site of entry
- Since the organisms can multiply in the host, fewer quantities are required to induce protection

- Fewer doses required
- Less chance of hypersensitivity
- Relatively cheap

Disadvantages

- Risk of **reversion** to **virulent form**
- Not safe in **immunosuppressed** individuals
- Proper storage is critical. Cold chain is required
- Risk of contamination with live organism
- Risk of spread to other animals

■ Examples

- ✓ Strains 19 and RB51 of *B. abortus* vaccine
- ✓ Render pest vaccine
- ✓ Anthrax vaccine
- ✓ African horse sickness vaccine, etc

Second generation vaccines (Subunit vaccines)

- Composed of semi-pure or purified antigens instead of whole organisms
- Consists of only those antigens that elicit protective immunity

Forms of subunit vaccines:

I) Inactivated exotoxins or toxoids

- Formaldehyde treated exotoxin is called toxoid
- Vaccination with toxoid induces antitoxoid antibodies, which are capable of binding to the toxin and neutralizing its effect
- Example: Tetanus and diphtheria vaccines

II) Capsular polysaccharide

- Capsular polysaccharides are plentiful on the bacterial surface and thus easy to purify
- Vaccination with vaccine consisting of purified capsular polysaccharide induces opsonizing antibodies, which coat the capsule and render the pathogen susceptible to phagocytosis by macrophage

Example: *Streptococcus pneumoniae* vaccine

III) Recombinant protein antigen

- Large quantities of purified immunogenic proteins (antigens) can be produced by recombinant DNA technology

- DNA coding for an Ag of interest is first isolated
- Then the DNA is inserted into a bacterium, yeast or mammalian cell
- The recombinant Ag then expressed
- The expressed Ag is used for vaccine development

Examples:

1. FMD vaccine (VP1 [protective antigen])
2. Feline leukemia vaccine (gp70 [protective antigen], commercially available)
3. Lyme vaccine: *Borrelia burgdorferi* (OspA [immunodominant outer surface lipoprotein])

Advantages

- Induce strong humoral and cell-mediated immune response
- They can safely be given to immunosuppressed individual
- Have an excellent safety profile
- Can be used in combination with other subunit vaccines

Disadvantages

- May require the use of adjuvants to enhance immune responses
- Antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface.

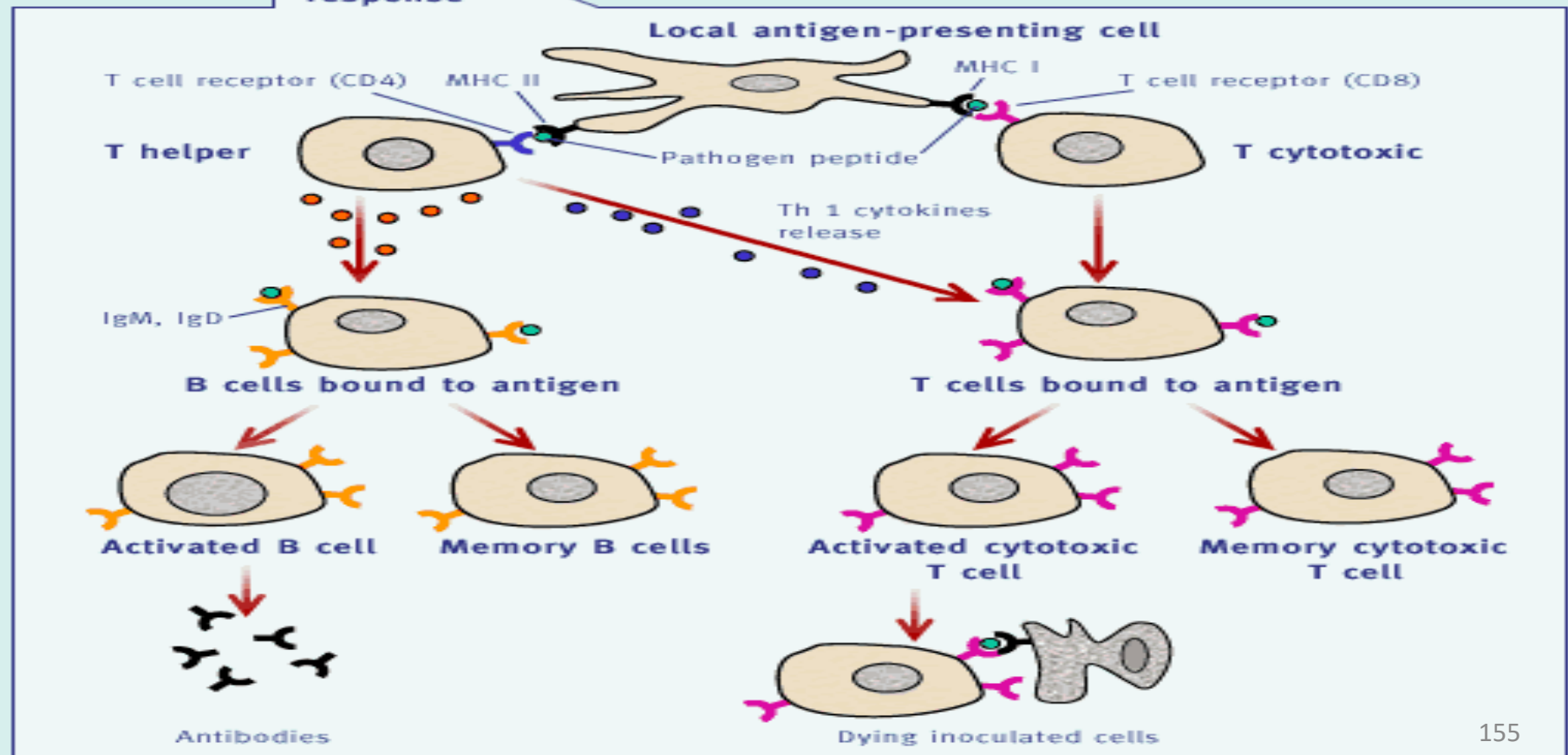
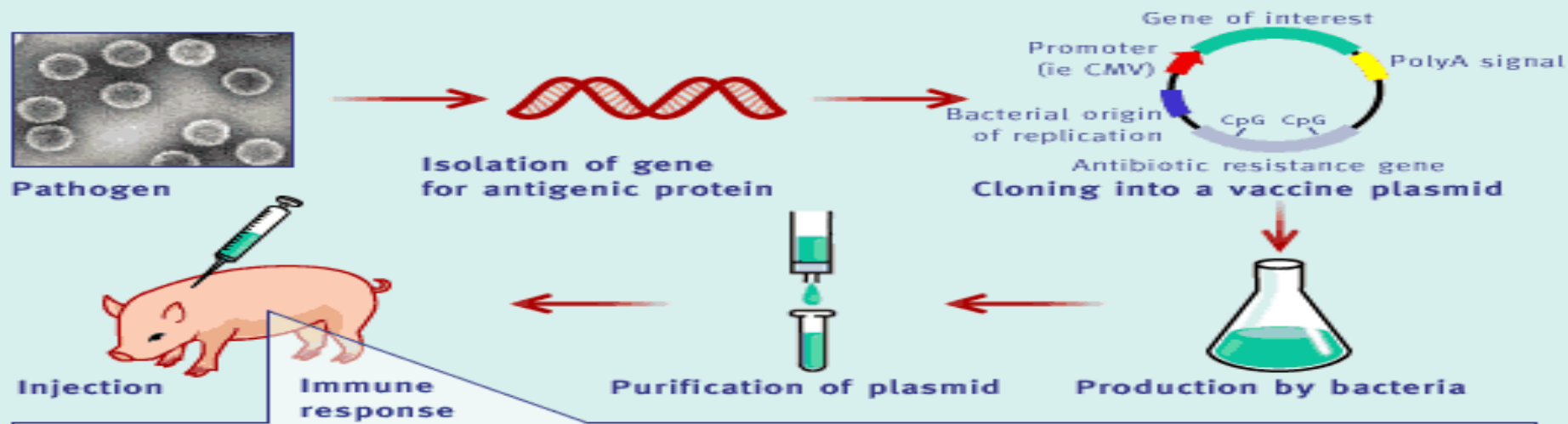
Third generation vaccines (DNA vaccine)

- **DNA vaccination**;- is the direct introduction of a bacterial plasmid DNA into host cells that expresses an antigenic protein under the control of a eukaryotic promoter./vector/
- As a consequence, the foreign antigen is expressed within the host cell and can simulate the induction of both humeral and cell-mediated immune responses

Delivery of DNA vaccine

- ✓ Intramuscular, intradermal or intranasal inoculation
- ✓ Particle-mediated intradermal delivery using a gene gun, in which the DNA is precipitated onto gold microspheres
- ✓ Using attenuated intracellular bacteria, such as *Shigella flexneri* or *Salmonella typhimurium*

Principle of DNA vaccination



Advantages

- Induce both humeral and cell-mediated immune responses
- The risks associated with live vaccines are absent
- Inexpensive and relatively easy to prepare
- There is no protein component and so there will be no immune response against the vector itself.

- Combination DNA vaccines containing the genes for multiple antigens of one pathogen, or single antigen from more than one pathogen, are also relatively easy to prepare
- DNA vaccines are very stable and therefore have a long shelf life and can be transported without a cold chain.

Disadvantages

- The plasmid persists for a long time, there is a potential for vaccine DNA to integrate into the host genome and activate oncogenesis or inhibit tumour suppressor genes
- The presence of an antibiotic-resistant gene in these plasmids also runs the risk of transferring this resistance to bacteria
- Large quantities of DNA are required to induce immune response
- Requires the use of adjuvants

Examples may include:

- FMD, Anthrax, TB, Influenza, Newcastle disease, Rabies, Canine parvovirus etc.
- DNA vaccines are in experiment

Table 4. DNA vaccines approved for veterinary use.

Type	Species	Target	Product/Company	License date/ country	Route of administration	Benefits
Prophylactic vaccine	Horses	West Nile Virus	West Nile-Innovator [®] / Fort Dodge Animal Health	2005 USA	IM ¹	Production of protective antibodies
Prophylactic vaccine	Salmon	Infectious haematopoietic necrosis virus (IHNV)	Apex-IHN [®] /Novartis Animal Health	2005 Canada	IM	Stimulation of innate and adaptive immune responses improving the welfare and product yield
Immunotherapy of cancer	Dogs	Melanoma	Oncept [™] /Merial	2010 USA	ID ² needle-free.	Production of antibodies capable of preventing the progress of the disease and prolonging the animal's life
Gene therapy	Swine	Growth hormone releasing hormone (GHRH)	LifeTide [®] SW5/VGX [™] Animal Health	2008 Australia	IM followed by electroporation	Decrease in perinatal mortality and morbidity, increase of the number of pigs weaned per sow and productivity ¹⁶⁰

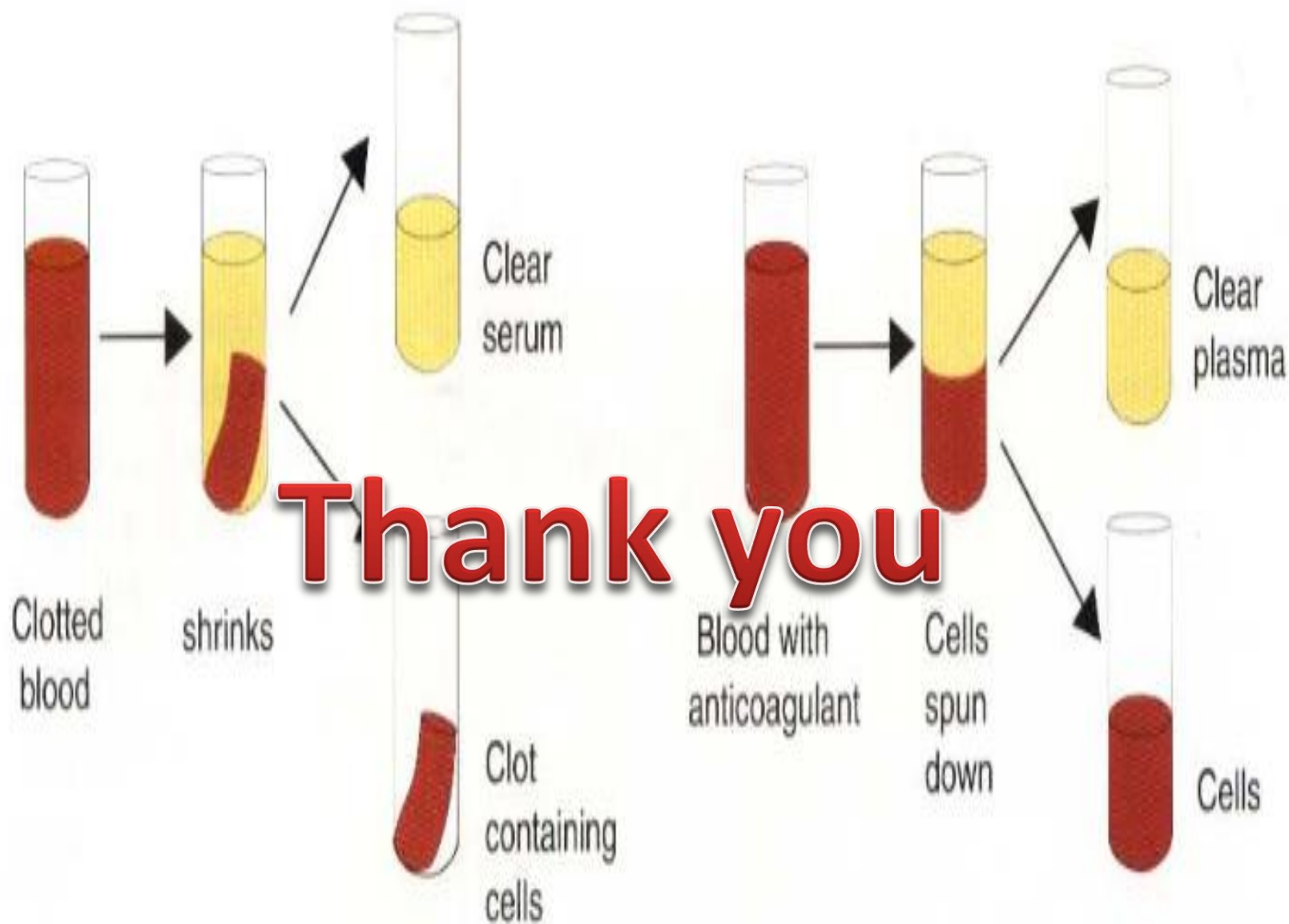


Figure 1-5. The difference between serum and plasma. Plasma contains blood-clotting components that are absent from serum.