UNIT – 4 Antigen – Antibody Reactions
(Antigen-Antibody Interactions In Vitro)

Antigens and antibodies combine with each other specifically and in observable manner. Antigen-Antibody reactions serve several purposes, which are as follows—

→ In the body, they form the basis of antibody-mediated immunity in infectious diseases, or of tissue injury in some types of hypersensitivity and autoimmune diseases.

→ In the laboratory, they help in the diagnosis of infections, in epidemiological surveys, in the identification of infectious agents and of non-infectious antigens such as enzymes.

→ In general, these reactions can be used for the detection and quantitation of either antigens or antibodies.

→ Antigen-Antibody reactions in vitro are known as **'Serological reactions'.**

Serology: The branch of medical immunology concerned with antigen-antibody reactions in vitro is **serology** [serum and -ology].

The reactions between antigens and antibodies occur in 3 stages—

1. **Primary stage**
2. **Secondary stage**
3. **Tertiary stage**

1. Primary stage: -

The primary stage is the initial interaction between antigen and antibody without any visible effects. This reaction is rapid, occurs even at low temperatures and obeys the general laws of physical chemistry and thermodynamics. The reaction is reversible.

2. Secondary stage: -

In this stage demonstrable events occur such as—

→ Precipitation

→ Agglutination

→ Lysis of cells

→ Killing of live antigens

→ Neutralization of toxins and other biologically active antigens

→ Fixation of complement

→ Immobilization of motile organisms

→ Enhancement of phagocytosis

3. Tertiary stage

Some antigen-antibody reactions occurring in vivo initiate chain reactions that lead to neutralization or destruction of injurious antigens, or to tissue damage. These are tertiary reactions and include humoral immunity against infectious diseases, clinical allergy and other immunological diseases.

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- ## General features of antigen-antibody reactions

1. The reaction is specific, an antigen combining only with its homologous antibody and vice versa. The specificity, however, is not absolute and 'cross reactions' may occur due to antigenic similarity or relatedness.
2. Entire molecules react and not fragment.
3. There is no denaturation of the antigen or the antibody during the reaction.
4. The combination occurs at the surface and is firm but reversible. The firmness of the binding is influenced by the affinity and avidity of the reaction. Affinity refers to the intensity of attraction between the antigen and antibody molecules. Avidity is the strength of the bond after formation of antigen-antibody complexes.
5. Both antigens and antibodies participate in the formation of precipitates or agglutinates.
6. Antigens and antibodies can combine in varying proportions, unlike chemicals with fixed valencies. Both antigens and antibodies are multivalent. Antibodies are generally bivalent, though some molecules may have 5 or 10 combining sites (IgA, IgM). Antigens may have valencies up to hundreds.

*** Measurement of antigen and antibody**

Many methods are available for the measurement of antigens and antibodies. Measurement may be in terms of **mass (e.g. mg Nitrogen)** or more commonly as **units or titre.**

 # Antibody titre: - It is the highest dilution of the serum which shows an observable reaction with the antigen in the particular test.

 # Sensitivity: - It is the ability of the test to detect even very minute quantities of antigen or antibody.

 # Specificity: - It is the ability of the test to detect reactions between homologous antigens and antibodies only, and with no other.

 # Types of antigen-antibody reactions

1. **Precipitation, Flocculation**
2. **Agglutination**
3. **Complement fixation**
4. **Neutralization**
5. **Opsonization**
6. **Imunofluorescence**
7. **Enzyme Linked Immunosorbent Assay (ELISA)**

1. Precipitation, Flocculation: -

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen-antibody complex forms an insoluble precipitate. The reaction is called as **Precipitation.**

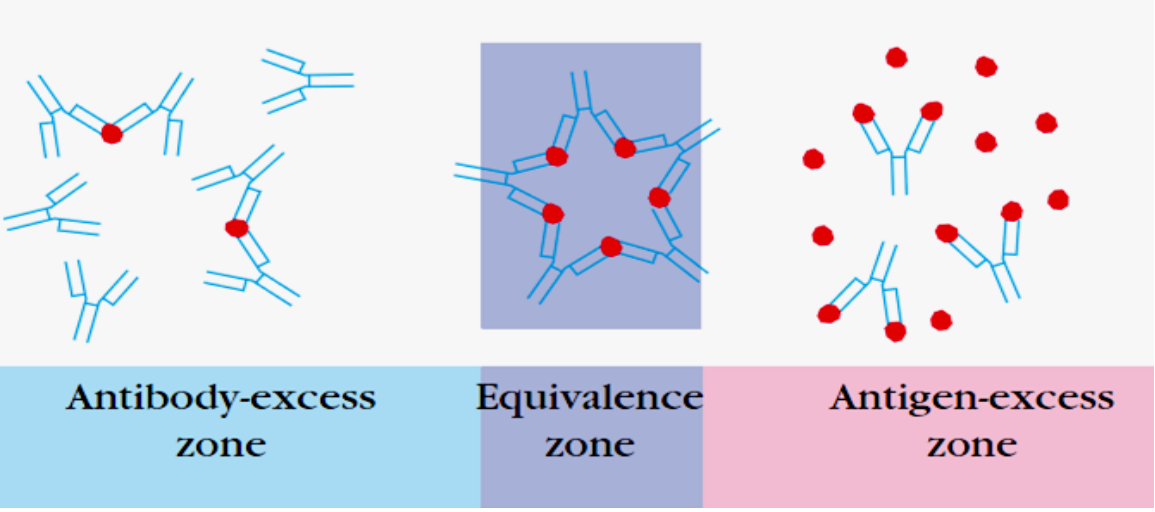
Flocculation: - When, instead of sedimenting, the precipitate remains suspended as floccules, the reaction is known as flocculation.

Precipitation can take place in liquid media or in gels such as agar, agarose or polyacrylamide.

The amount of precipitate formed is greatly influenced by the relative proportions of antigens and antibodies. If increasing quantities of antigens are added to the same amount of antiserum in different tubes, precipitation will be found to occur most rapidly and abundantly in one of the middle tubes in which the antigen and antibody are present in optimal or equivalent proportions.

- **Mechanism of precipitation [The Lattice hypothesis of Marrack]**

According to this hypothesis, multivalent antigens combine with bivalent antibodies in varying proportions, depending on the antigen-antibody ratio in the reacting mixture. Precipitation results when a large lattice is formed consisting of alternating antigen and antibody molecules. This is possible only in the zone of equivalence. In the zones of antigen or antibody excess, the lattice does not enlarge, as the valencies of the antibody and the antigen, respectively, are fully satisfied. The lattice hypothesis holds good for agglutination also.



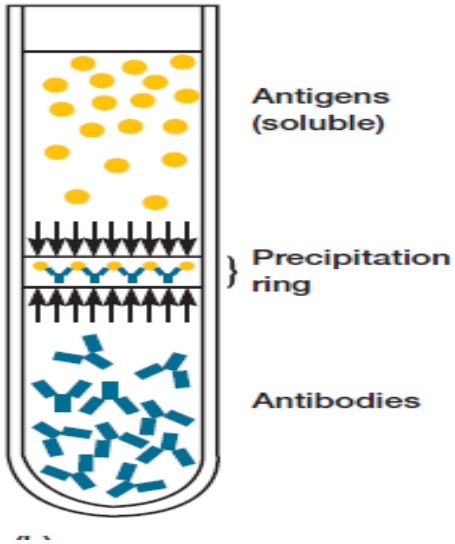
*** Applications of precipitation reaction**

1. The precipitation test may be carried out as either a qualitative or quantitative test.

It is very sensitive in the detection of antigens but relatively less sensitive for the detection of antibodies and as little as 1 μg of Antigen can be detected by precipitation tests.

2. **Forensic applications: -** It is used in the identification of blood and seminal stains in murder case and rape case. Also it is used in food adulterations.
3. **Ring test:** - This is the simplest type of precipitation test, consists of layering the antigen solution over a column of antiserum in a narrow tube. E.g. Grouping of streptococci by the Lancefield technique.
4. **Slide test (VDRL Test): -** When a drop each of antigen and antiserum are placed on a slide and mixed by shaking, floccules appear. The VDRL test for syphilis is an example of flocculation.

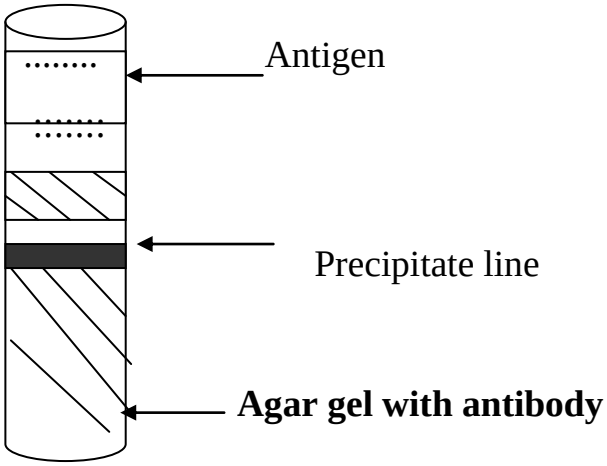
5. **Tube test: -** A quantitative tube flocculation test is used for the standardization of toxins and toxoids. Serial dilutions of the toxin/toxoid are added to the tubes containing a fixed quantity of the antitoxin. The amount of toxin or toxoid that flocculates optimally with one unit of the antitoxin is defined as an Lf dose.



6. **Immunodiffusion (Precipitation in gel): -** There are several advantages of precipitation in gel rather than in liquid medium. The reaction is visible as a distinct band of precipitation, which is stable and can be stained for preservation. As each antigen-antibody reaction gives rise to a line of precipitation, the number of different antigens in the reacting mixture can be readily observed. Immunodiffusion also indicates identity, cross-reaction and non-identity between different antigens.
Immunodiffusion is usually performed in a soft (1 %) agar gel. Different modifications of immunodiffusion are as follows—

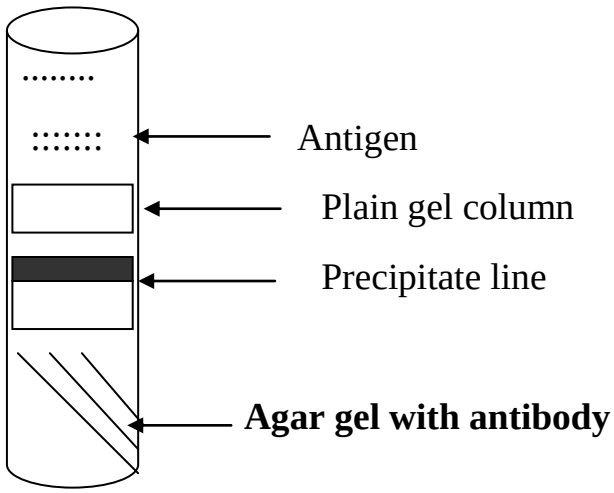
A) Single diffusion in one dimension (Oudin procedure): -

The antibody is incorporated in agar gel in a test tube and the antigen solution is layered over it. The antigen diffuses downward through the agar gel, forming a line of precipitation. The number of bands indicates the number of different antigens present.



B) Double diffusion in one dimension (Oakley-Fulthorpe procedure):

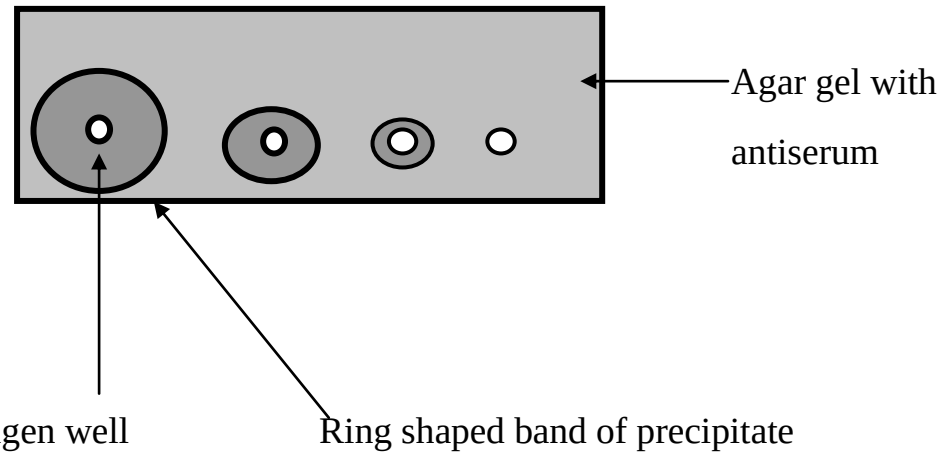
Here, the antibody is incorporated in gel, above which is placed a column of plain agar. The antigen is layered on top of this. The antigen and antibody move towards each other through the intervening column of plain agar and form a band of precipitate where they meet at optimum proportion.



C) Single diffusion in two dimensions (Radial immunodiffusion)

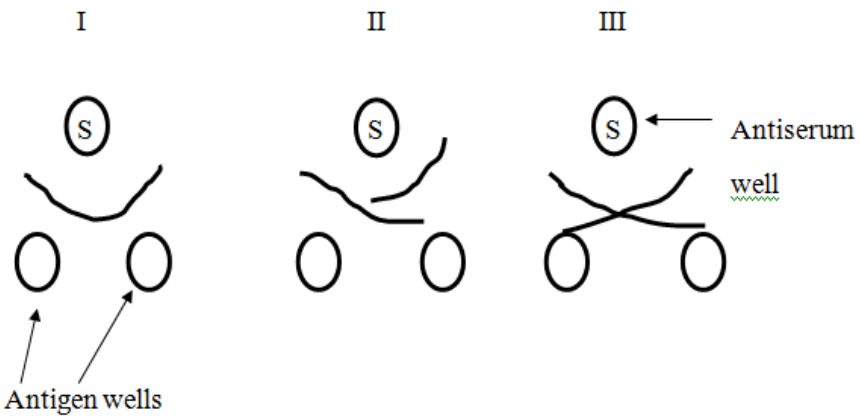
Here the antiserum is incorporated in agar gel poured on a flat surface (Slide or petridish). The antigen is added to the wells cut on the surface of the gel. It diffuses radilly from the well and forms ring shaped bands of precipitation (halos) concentrically around the well. The diameter of the halo gives an estimate of the concentration of the antigen.

This method is used for the estimation of the immunoglobulin classes in sera and for screening sera for antibodies to influenza viruses, among others.



D) Double diffusion in two dimensions (Ouchterlony procedure)

This is the immunodiffusion method most widely used and helps to compare different antigens and antisera directly. Agar gel is poured on a slide and wells are cut using a template. The antiserum is placed in the central well and different antigens in the surrounding wells. If two adjacent antigens are identical, the lines of precipitate formed by them will fuse. If they are unrelated, the lines will cross each other.

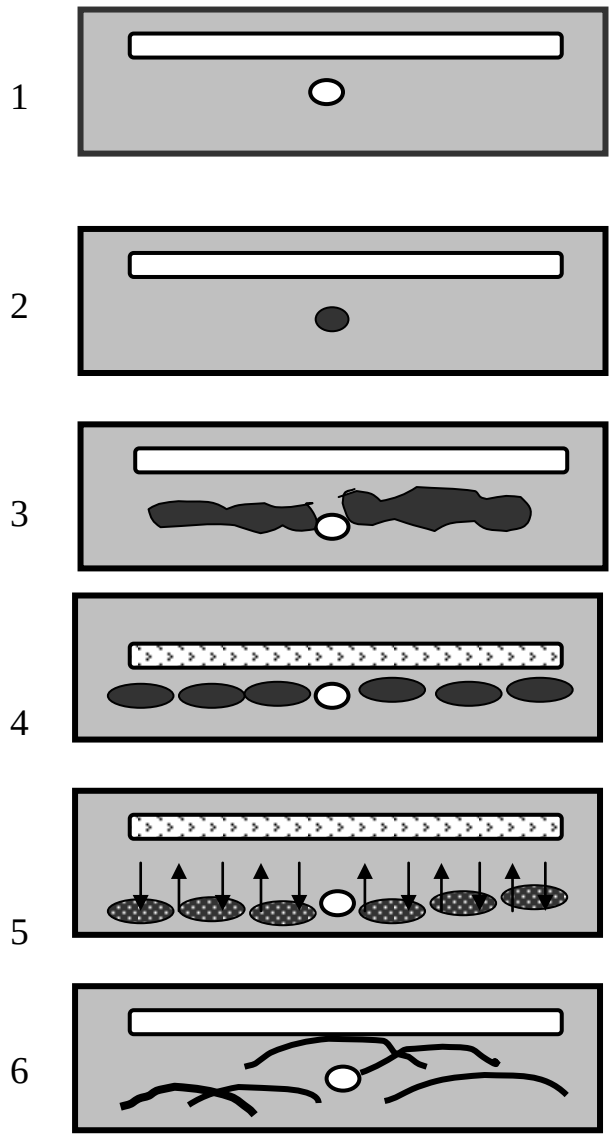


(I – Partial identity, II & III – Lack of relatedness)

7) Immunoelectrophoresis: -

This involves the electrophoretic separation of a composite antigen (such as serum) into its constituent proteins, followed by immunodiffusion against its antiserum, resulting in separate precipitin lines, indicating reaction between each individual protein with its antibody. This enables identification and approximate quantitation of the various proteins present in the serum.

The technique is performed on agar or agarose gel on a slide, with an antigen well and an antibody trough cut on it. The test serum is placed in the antigen well and electrophoresed for about an hour. Antibody against human serum is then placed in the trough and diffusion allowed to proceed for 18-24 hours. The resulting precipitin lines can be photographed and the slides dried, stained and preserved for record. Over 30 different proteins can be identified by this method in human serum. This is useful for testing for normal and abnormal proteins in serum and urine.



1. Semisolid agar layered on the glass slide. A well for antigen and trough for antiserum cut out of agar.

2. Antigen well filled with human serum.

3. Serum separated by electrophoresis.

4. Antiserum trough filled with antiserum to whole human serum.

5. Serum and antiserum allowed to diffuse into agar.

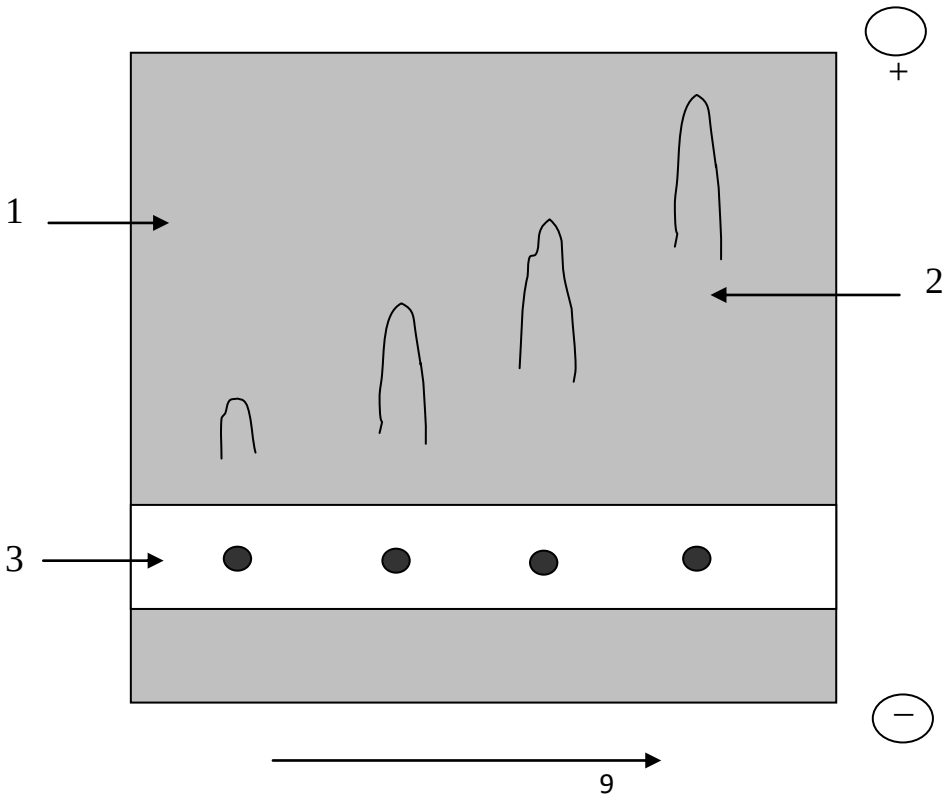
6. Precipitin lines form for individual serum proteins.

8) Electroimmunodiffusion: -

The development of precipitin lines can be speeded up by electrically driving the antigen and antibody. Various methods have been described combining electrophoresis with diffusion.

I) One-dimensional single electroimmunodiffusion (Rocket electrophoresis)

The main application of this technique is for quantitative estimation of antigens. The antiserum to the antigen is incorporated in agarose and spread on the glass slide. The antigen is placed in wells punched in the gel in increasing concentrations. The antigen is then electrophoresed into the antibody containing agarose. The pattern of immunoprecipitation resembles a rocket and hence the name.



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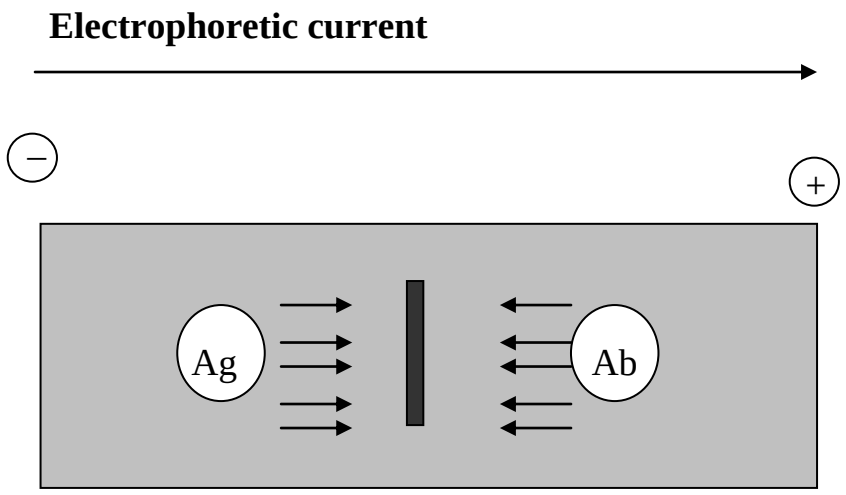
Rocket electrophoresis

1 -- Antibody in agarose gel
2 -- Precipitation arcs
3 -- Antigen wells
4 -- Increasing antigen concentration

II) One dimensional double electroimmunodiffusion: -

(Counterimmunoelectrophoresis, CIE)

This involves simultaneous electrophoresis of the antigen and antibody in gel in opposite directions resulting in precipitation at a point between them. This method produces visible precipitation lines within 30 minutes. The clinical applications are for detecting various antigens such as specific antigens of cryptococcus and meningococcus in the cerebrospinal fluid.



Counterimmunoelectrophoresis (CIE)

Antigen and antibody are driven together by an electric current and a precipitin line forms.

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2. Agglutination reaction

1. When a **particulate** antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.

2. Agglutination is more sensitive than precipitation for detection of antibodies.

3. The same principles govern agglutination and precipitation. Agglutination occurs optimally when antigens and antibodies react in equivalent proportions. The zone phenomenon may be seen when either an antibody or an antigen is in excess.

4. Applications of agglutination reaction: -

I) Slide agglutination: -

When a drop of the appropriate antiserum is added to a smooth, uniform suspension of a particulate antigen in a drop of saline on a slide or tile, agglutination takes place. A positive result is indicated by the clumping together of the particles. The reaction is facilitated by mixing the antigen and the antiserum with a loop or by gently rocking the slide. Depending upon the titre of the serum, agglutination may occur instantly or within seconds.

Agglutination is usually visible to the naked eye but may sometimes require confirmation under the microscope.

e.g. Blood group determination and cross matching.

Identification of many bacterial isolates.

Blood group determination
Requirements: -

i) Clean grease free slide
ii) Sterile needle or lancet
iii) Absolute alcohol
iv) Cotton, pins
v) Anti-A, Anti-B and Anti-D (Anti Rh) antiserum

Procedure: -

i) Sterile finger of left hand with alcohol.
ii) Prick it with sterile lancet and wipe out 1 to 2 drops.
iii) Take drops of blood at 3 places on a clean grease free slide.
iv) Add Anti-A, Anti-B and Anti-D antiserum on first, second and third drop of blood respectively.
v) Mix all drops with separate pins.

Observation: -

Observe for agglutination in each drop.

Result: -

Sr. No	Agglutination in Anti-A added drop	Agglutination in Anti-B added drop	Agglutination in Anti-D added drop	Blood group
1	**Present**	**Absent**	**Present**	**A Positive**
2	**Present**	**Absent**	**Absent**	**A Negative**
3	**Absent**	**Present**	**Present**	**B Positive**
4	**Absent**	**Present**	**Absent**	**B Negative**
5	**Present**	**Present**	**Present**	**AB Positive**
6	**Present**	**Present**	**Absent**	**AB Negative**
7	**Absent**	**Absent**	**Present**	**O Positive**
8	**Absent**	**Absent**	**Absent**	**O Negative**

II) Tube agglutination

This is the standard quantitative method for the measurement of antibodies. When a fixed volume of a particulate antigen suspension is added to an equal volume of serial dilutions of an antiserum in test tubes, the agglutination titre of the serum can be estimated.

E.g. Diagnosis of typhoid by Widal test

III) The antiglobulin test (Coombs test) (Haemolytic disease of the newborn)

This test was devised by Coombs, Mourant and Race (1945) for the detection of anti-Rh antibodies that do not agglutinate Rh-positive RBCs in saline.

When sera containing incomplete anti-Rh antibodies are mixed with Rh-positive RBCs, the antibody globulin coats the surface of the RBCs, but they are not agglutinated. When such RBCs coated with the antibody globulin treated with a rabbit antiserum against human gammaglobulin **(antiglobulin or Coombs serum),** the RBCs are agglutinated.

E.g. Detection of haemolytic disease of the newborn due to Rh incompatibility.

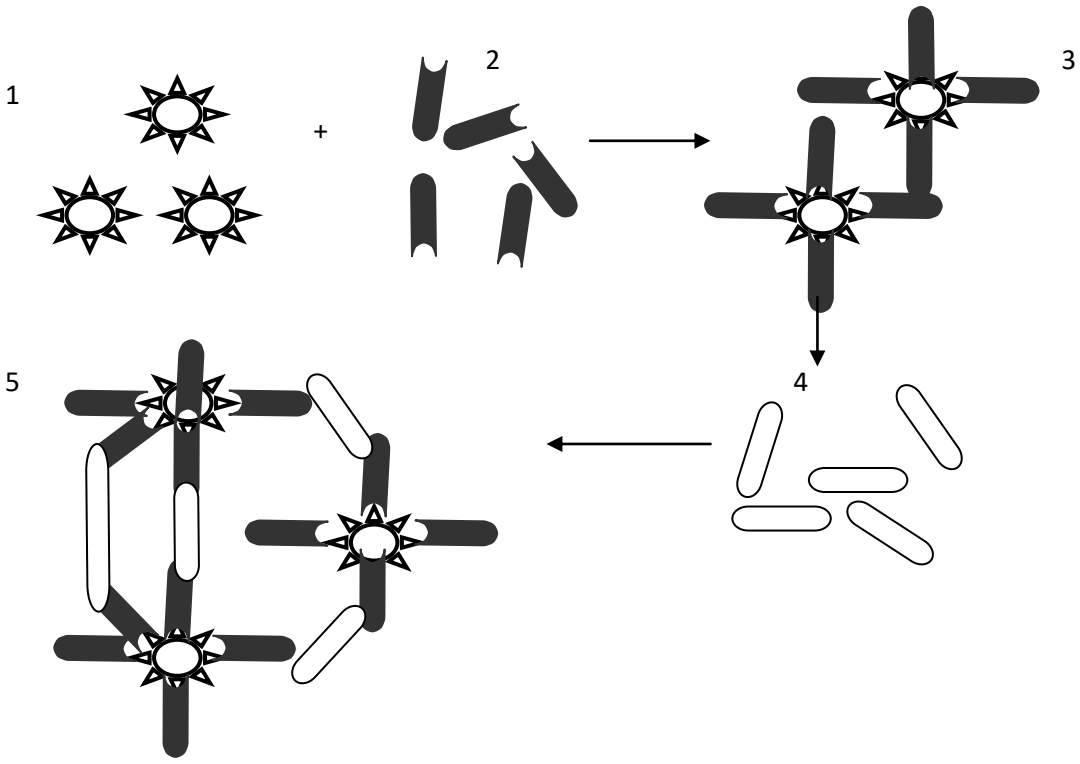
RBCs of newborn are washed to become free from unattached protein and then mixed with a drop of Coombs serum, agglutination results.

1. Rh positive RBCs are mixed with incomplete antibody

2. The antibody coats the RBCs

3. Antibodies being incomplete, cannot produce agglutination

4. On addition of antiglobulin serum, which is complete antibody to immunoglobulin, agglutination takes place.



IV) Passive agglutination test (RA factor determination test)/ Rose-Waller test: -

The only difference between the requirements for the precipitation and agglutination tests is the physical nature of the antigen. By attaching soluble antigens to the surface of carrier particles, it is possible to convert precipitation test into agglutination test. Such tests are known as passive agglutination tests.

The commonly used carrier particles are RBCs, Polystyrene Latex particles (0.8 – 1 mm in diameter) or Bentonite.

E.g. **Rose-Waller test** (RA factor determination test)

In Rheumatoid Arthritis, an auto-antibody (RA factor) appears in the serum, which acts as an antibody to gammaglobulin. The RA factor is able to agglutinate RBCs coated with globulins. The antigen used for this test is suspension of sheep RBCs sensitized with a dose of rabbit anti-sheep RBC antibody **(Amboceptor).**

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3. Complement fixation test (CFT) / Wasserman test for syphilis

1. Complement takes part in many immunological reactions and is absorbed during the combination of antigens with their antibodies.

2. In the presence of the appropriate antibodies, complement lyses RBCs, Bacteria, immobilizes motile microorganisms, promotes phagocytosis and contributes to tissue damage in certain types of hypersensitivity.

3. The ability of antigen-antibody complex to **'fix'** complement is made use of in the complement fixation test **(CFT).**

4. This is a very versatile and sensitive test, applicable with various types of antigens and antibodies and capable of detecting as little as 0.04 mg of antibody nitrogen and 0.1 mg of antigen.

5. Requirements: -

i) Antigen (May be soluble or particulate)

ii) Antibody / Antiserum (Should be heated at 56^{0} C to destroy complement activity.

iii) Complement (Taken from guinea pig serum).

iv) Sheep RBCs

v) Amboceptor (Rabbit antibody to sheep RBCs)

vi) Physiological saline with added calcium and magnesium ions.

vii) Each of these reagents has to be separately standardized.

viii) Example **Wasserman test for syphilis.**

Procedure: -

Step – I

The inactivated serum of the patient is incubated at 37 ^{0} c for one hour with Wasserman antigen and a fixed amount (two units) of guinea pig complement.

If the serum contains syphilitic antibody, the complement will be utilized during the antigen-antibody interaction and will be fixed.

If the serum does not contain the antibody, no antigen-antibody reaction occurs and the complement will not be fixed.

Step – II

Whether the complement is fixed or not is detected in this step. It consists of addition of sheep RBCS and amboceptor and incubation at 37 ^{0} C for 30 minutes.

Result: -

Positive test: - One drop of reaction mixture is taken on slide and observed under microscope. Absence of lysis of RBCs indicates that the complement was used up in the first step and, therefore, the serum contained the antibody.

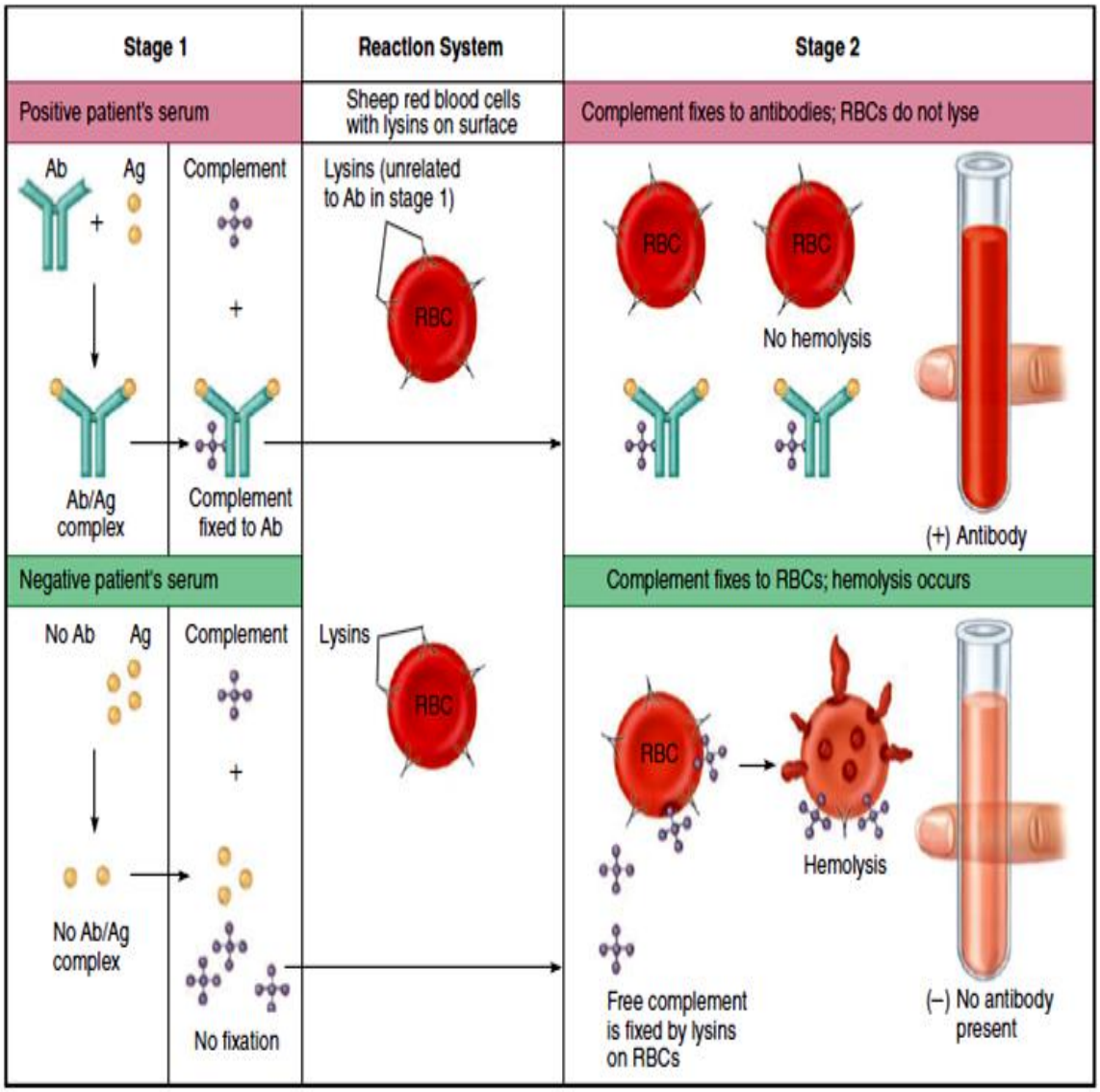
Negative test: - Lysis of RBCs indicates that complement was not fixed in the first step and, therefore, the serum did not have the antibody.

I) ANTIGEN + TEST SERUM (Contains Antibody) + COMPLEMENT + HEMOLYTIC SYSTEM (Sheep RBCs + Amboceptor) -- Result – No haemolysis -- Complement Fixed

CF TEST POSITIVE

II) ANTIGEN + TEST SERUM (Contains NO Antibody) + COMPLEMENT + HEMOLYTIC SYSTEM (Sheep RBCs + Amboceptor) -- Result – Haemolysis -- Complement Not Fixed

CF TEST NEGATIVE



Complement fixation test. In this example, two serum samples are being tested for antibodies to a certain infectious agent. In reading this test, one observes the cloudiness of the tube. If it is cloudy, the RBCs are not hemolyzed and the test is positive. If it is clear and pink, the RBCs are hemolyzed and the test is negative.

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4. NEUTRALIZATION TESTS

Specific antibodies are able to neutralize the biological effects of viruses, toxins and enzymes.

Virus neutralization tests: Neutralization of viruses by their antibodies can be demonstrated in various systems, Neutralization of bacteriophages can be demonstrated by the plaque inhibition test. When bacteriophagcs are seeded in appropriate dilution on lawn cultures of susceptible bacteria, plaques of lysis are produced. Specific antiphage serum inhibits plaque formation. Neutralization of animal viruses can be demonstrated in three systems — animals, eggs and tissue culture.

Toxin neutralization: Bacterial exotoxins are good antigens and induce the formation of neutralizing antibodies (antitoxins) which are important clinically, in protection against and recovery from diseases such as diphtheria and tetanus. The toxicity of endotoxins is not neutralized by anti-sera.

Toxin neutralization can be tested *in vivo* or *in vitro*. Neutralization tests in animals consist of injecting into them toxin antitoxin mixtures and estimating the least amount of antitoxin that prevents death or disease in the animal. With the diphtheria toxin, which in small doses causes a cutaneous reaction, neutralization tests can be done on the human skin. **The Schick test** is based on the ability of circulating antitoxin to neutralize the diphtheria toxin given intradermally, and indicates the immunity or susceptibility to the disease.

Toxin neutralization ***in*** *vitro* depends on the inhibition of some demonstrable toxic effect. An example is the **antistreptolysin O test,** in which antitoxin present in patient's sera neutralizes the haemolytic activity of the streptococcal O haemolysin.

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5. IMMUNOFLUORESCENCE / Coons Test

Fluorescence is the property of absorbing light rays of one particular wavelength and emitting rays with a different wavelength. Fluorescent dyes show up brightly under ultraviolet light as they convert ultraviolet into visible light. Coons and his colleagues (1942) showed that fluorescent dyes can be conjugated to antibodies and that such 'labelled' antibodies can be used to locate and identify antigens in tissues. This 'fluorescent antibody' or immunofluorescence technique has several diagnostic and research applications.

Direct immunofluorescence test: In its simplest form, it can be used for the identification of bacteria, viruses or other antigens, using the specific antiserum labelled with a fluorescent dye. For example, direct immunofluorescence is now

routinely used as a sensitive method of diagnosing **rabies,** by detection of the rabies virus antigens in brain smears. A disadvantage of this method is that separate fluorescent conjugates have to be prepared against each antigen to be tested.

Indirect immunofluorescence test overcomes this difficulty by using an antiglobulin fluorescent conjugate. An example is the fluorescent treponemal antibody test for the diagnosis of syphilis. Here a drop of the test serum is placed on a smear of *T. pallidum* on a slide and after incubation, the slide is washed well to remove all free serum, leaving behind only antibody globulin, if present, coated on the surface of the treponemes. The smear is then treated with a fluorescent labelled antiserum to human gammaglobulin. The fluorescent conjugate reacts with antibody globulin bound to the treponemes. After washing away all the unbound fluorescent conjugate, when the slide is examined under ultraviolet illumination, the treponemes will be seen as bright objects against a dark background, if the test is positive. If the serum does not have anti treponemal antibody, there will be no globulin coating on' the treponemes and, therefore, they will not take on the fluorescent conjugate. A single antihuman globulin fluorescent conjugate can be employed for detecting human antibody to any antigen.

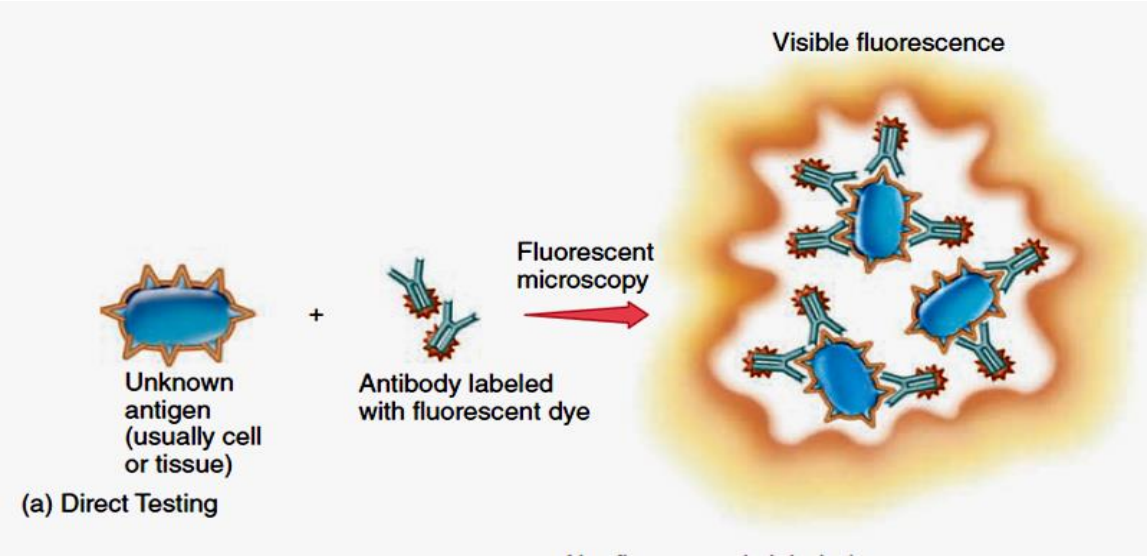
Fluorescent dyes may also be conjugated with complement. Labelled complement is a versatile tool and can be employed for the detection of antigen or antibody. Antigens also take fluorescent labelling, but not as well as antibodies do.

Sandwich technique

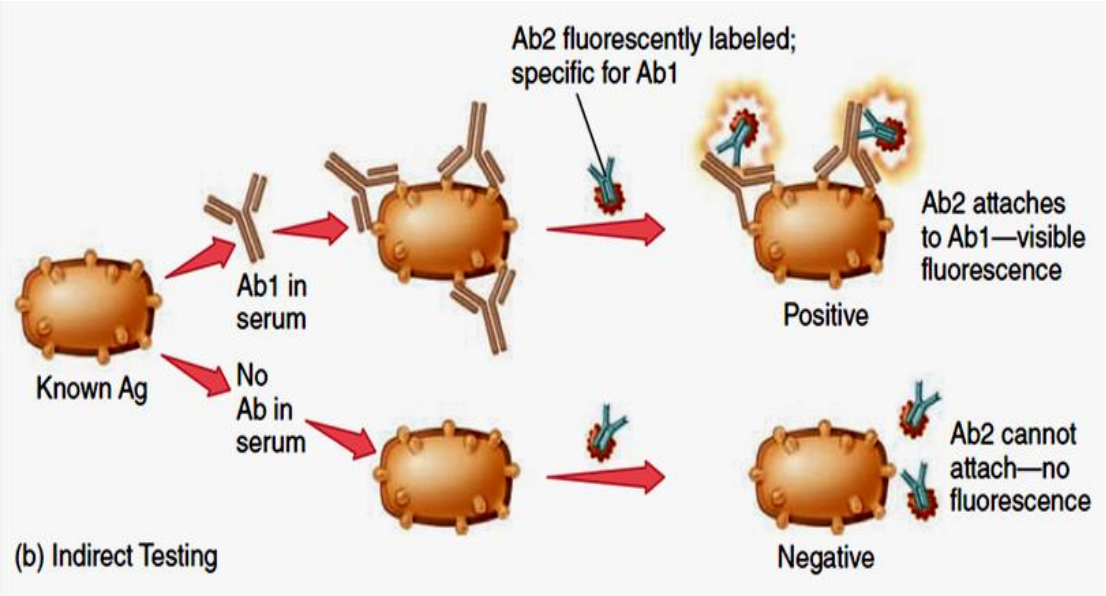
For detection of antibodies by immunofluorescence, the 'sandwich' technique can be employed. The antibody is first allowed to react with unlabelled antigen, which is then treated with fluorescent labelled antibody, thus forming a sandwich, the antigen being in the middle, with labelled and unlabelled antibody on either side.

Fluorescent dyes

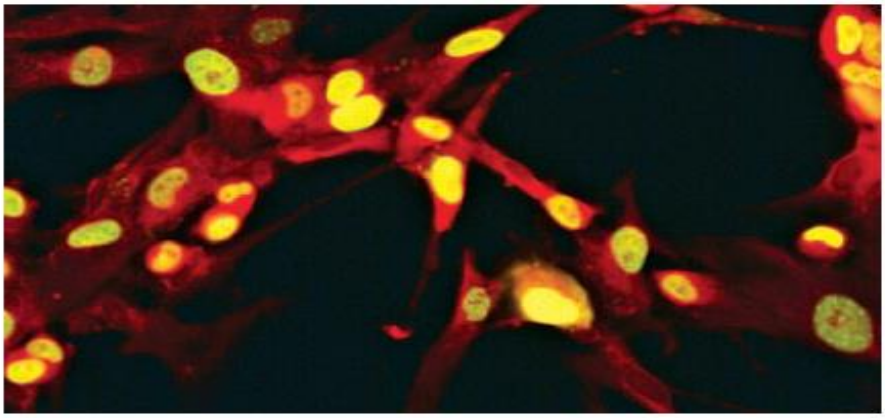
The fluorescent dyes commonly used are fluorescein isothiocyanate and lissamine-rhodamine, exhibiting blue-green and orange-red fluorescence, respectively. By combining the specificity of serology with the localizing capacity of histology, immunofluorescence helps in the visualization of antigen antibody reactions *in situ.* The major disadvantage of the technique is the frequent occurrence of nonspecific fluorescence in tissues and other materials.



Immunofluorescent testing. (a) Direct: Unidentified antigen (Ag) is directly tagged with fluorescent Ab.



(b) Indirect: Ag of known identity is used to assay unknown Ab; a positive reaction occurs when the second Ab (with fl uorescent dye) affixes to the first Ab.



(c) Indirect Immunofluorescence of specimen

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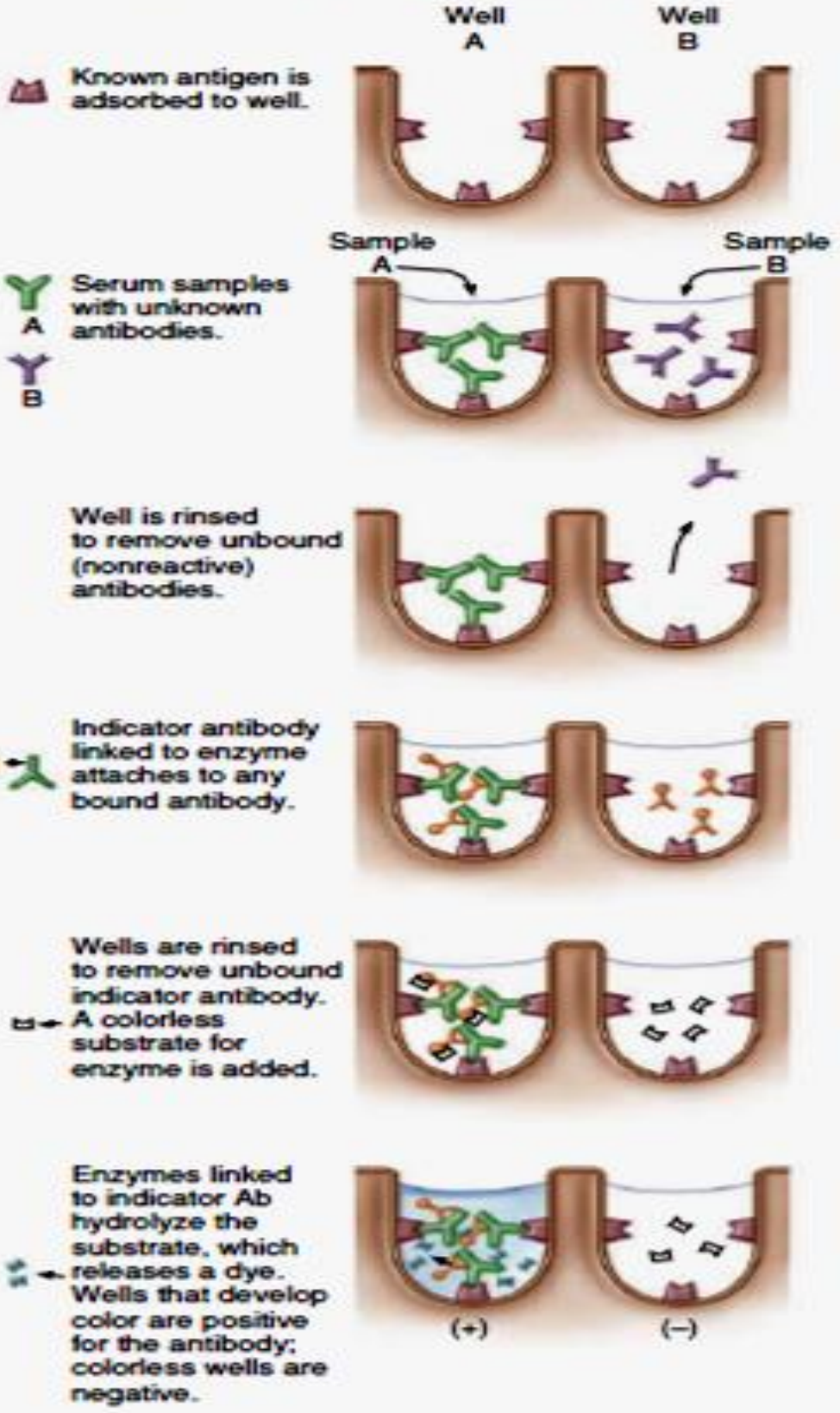
6. Enzyme-linked immunosorbent assay (ELISA)

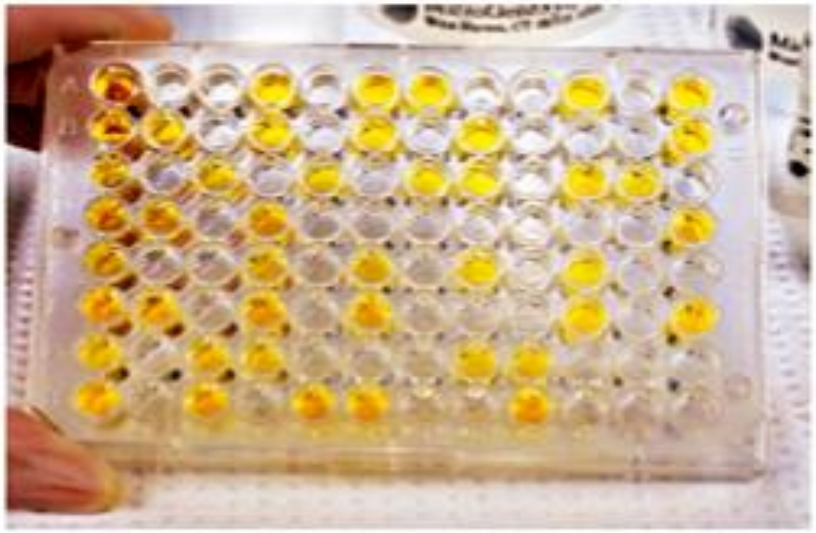
This is a simple and versatile technique, which is as sensitive as radioimmunoassay and needs only microlitre quantities of test reagents. ELISA has found application for the detection of a variety of antibodies and antigens, such as hormones, toxins and viruses. The test may be done in polystyrene tubes (macro-ELISA) or polyvinyl microtitre plates (micro-ELISA). The principle of the test can be illustrated by outlining its application for the detection of the rotavirus antigen in faeces.

The wells of a microtitre plate are coated with goat antirotavirus antibody. After thorough washing, the faecal samples to be tested are added and incubated overnight at 4°C or for 2 hours at 37°C. Suitable positive and negative controls are also put up. The wells are washed and guinea pig antirotavirus antiserum, labelled with alkaline phosphatase, added and incubated at 37°C for one hour. After washing, a suitable substrate (paranitrophenyl phosphate) is added and held at room temperature till the positive controls show the development of a yellow colour. The phosphatase enzyme splits the substrate to yield a yellow compound. If the test sample contains rotavirus, it is adsorbed to the antibody coating the wells. When the enzyme-labelled antibody is added subsequently, it is in turn adsorbed. The presence of residual enzyme activity, indicated by the development of yellow colour, therefore, denotes a positive test. If the sample is negative, there is no **colour** change.

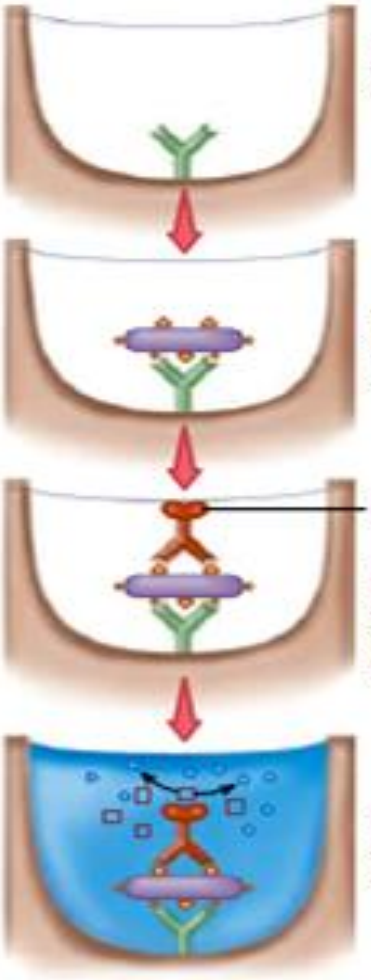
(a) **Indirect ELISA**, comparing a positive vs. negative reaction. This is the basis for HIV screening tests.



(b) **Microtiter ELISA Plate with 96 Tests for HIV Antibodies**
Colored wells indicate a positive reaction.



(c) **Capture or Antibody Sandwich ELISA Method.**
Note that an antigen is trapped between two antibodies. This test is used to detect the measles virus.



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General methods of prophylaxis.

Many issues are involved contained by the prevention of microbial diseases.

1. **Immunoprophylaxis** against microbial illnesses includes the use of vaccines or antibody-containing preparations to provide a susceptible individual with immunologic protection against a specific disease. Immunization against microbial illnesses can be either active or passive. With active immunity, protection is achieved by stimulating the body's immune system to produce its own antibodies by immunization with a virus preparation. Passive immunity is conferred by administering antibodies formed in another host. For example, an antibody-containing gamma globulin preparation may protect a susceptible individual exposed to a microbial illness.

2. Avoidance of microbial exposure. This is an effective means of preventing the transmission of HIV-1, which is spread through sexual contact and exposure to blood of infected individuals. Blood bank testing, e.g., for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, HTLV-I, and hepatitis C, also avoids exposure by identifying and discarding blood units contaminated with these infectious agents.

3. Control of nonhuman microbial reservoirs: The most notable success was the control and elimination of rabies in some countries through removal of stray dogs, quarantine of incoming pets, and vaccination of domestic animals.

4. Vector control: Transmission of viral disease by the bite of an arthropod vector, yellow fever was transmitted by mosquitoes, malaria by mosquitoes, gastro by flies etc. These procedures include draining swamps, applying insecticide, screening homes, and using insect repellant or protective clothing.

5. Improvement in sanitation: The well-known link between the discharge of raw sewage into tidal waters, contamination of shellfish, and type A hepatitis is an example of a situation readily reversible by improved sanitary practices.

Toxoids

A **toxoid** is a bacterial toxin (usually an exotoxin) whose toxicity has been inactivated or suppressed either by chemical (formalin) or heat treatment, while other properties, typically immunogenicity, are maintained. Thus, when used during vaccination, an immune response is mounted and immunological memory is formed against the molecular markers of the toxoid without resulting in toxin-induced illness.

E. G. There are toxoids for prevention of diphtheria, tetanus and botulism.

Toxoids are used as vaccines because they induce an immune response to the original toxin or increase the response to another antigen since the toxoid markers and toxin markers are preserved.

For example, the tetanus toxoid is derived from the tetanospasmin produced by *Clostridium tetani* which causes tetanus. Botulin is produced by *Clostridium botulinum* and it causes the deadly disease botulism.

While patients may sometimes complain of side effects after a vaccine, these are associated with the process of mounting an immune response and clearing the toxoid, not the direct effects of the toxoid. The toxoid does not have virulence, as did the toxin before inactivation.

Preparation

The *Corynebacterium diphtheria* and *Clostridium tetani* organisms are grown on modified Mueller's media 1, 2 which contains bovine extracts. Tetanus and diphtheria toxins produced during growth of the cultures are detoxified with formaldehyde. The detoxified materials are then separately purified by ammonium sulfate fractionation. The diphtheria toxoid is further purified by column chromatography. The tetanus and diphtheria toxoids are individually adsorbed onto aluminum phosphate.

Each 0.5 ml dose is formulated to contain the following active ingredients: 2 Lf of tetanus toxoid and 2 Lf of diphtheria toxoid. Each 0.5 ml dose also contains aluminum adjuvant (not more than 0.53 mg aluminum by assay), < 100 mcg (0.02%) of residual formaldehyde, and a trace amount of thimerosal [mercury derivative, (<0.3 mcg mercury/dose)] (not as a preservative) from the manufacturing process.

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Immune Sera

The serum of an animal or human containing antibodies against a specific disease, used to confer passive immunity to that disease. Antisera do not provoke the production of antibodies.

There are two types of antisera:

Antitoxin neutralizes the toxin produced by specific bacteria but does not kill the bacteria.

Antimicrobial serum acts to destroy bacteria by making them more susceptible to leukocytic action. Polyvalent antiserum acts on more than one antigenic determinant; monovalent antiserum acts on only one.

The resistance is transferred passively to a recipient by administration of **antibodies.** The agents used for this purpose are **Hyper Immune Sera (HIS)** of animal or human. Hyper immune sera are prepared by active hyper immunization in horses using the appropriate antigen.

e.g. Anti Tetanus Serum, Anti Botulism Serum, Anti Venom Serum, Anti Rabies Serum.

e.g. **Anti Tetanus Serum (ATS)** used for passive immunization against tetanus is prepared by administrating a series of doses of tetanus toxoid to horses, bleeding them and separating the serum. The antibodies in the serum are concentrated & purified by fractionation and enzyme treatment. The preparation is standardized to contain an adequate number of units of **antitoxin per ml.**

ATS is administered parenterally and subcutaneously for prophylaxis and intravenously for treatment. ATS provides an immediate supply of the antitoxin in the recipient's circulation.

Passive immunization is indicated for providing immediate & temporary protection in a non-immunized host, when there is insufficient time for active immunization to take effect. It is also indicated for treatment of infections.

Adjuvants

The term adjuvant refers to any substance that enhances the immunogenecity of an antigen. Adjuvants may confer immunogenecity on nonantigenic substances, increase the concentration and persistence of the circulating antibody, induce or enhance the degree of cellular immunity and lead to the production of 'adjuvant disease' such as allergic disseminated encephalomyelitis. Many substances exhibit adjuvant activity.

To enhance the immune response to an immunogen, various additives called adjuvants can be used. When mixed and injected with an immunogen, an adjuvant will enhance the immune response. An adjuvant is not a substitute for a carrier protein because it enhances the immune response to immunogens but cannot itself render haptens immunogenic. Adjuvants are nonspecific stimulators of the immune response, helping to deposit or sequester the injected material and causing a dramatic increase in the antibody response.

Repository adjuvants

Such as **Aluminium hydroxide or Phosphate.**

Complete Freund's adjuvant (CFA or FCA).

This reagent consists of a water-in-oil emulsion and killed *Mycobacterium*. The oil-and-water emulsion localizes the antigen for an extended period of time, and the *Mycobacterium* attracts macrophages and other appropriate cells to the injection site. Complete Freund's adjuvant is used for the first injection (immunization). Subsequent boosts use immunogen in an emulsion incomplete Freund's adjuvant (IFA or FIA), which lacks the *Mycobacterium*. Freund's adjuvants are very effective, but they do pose risks to both animal and researcher because of the toxic mycobacterial components.

Besides increasing the humoral immune response, it induces a high degree of cellular immunity (delayed hypersensitivity) as well. As it produces a local granuloma, it is unsuitable for human use.

Others such as silica particles, Beryllium sulphate and endotoxins

These activate macrophages.

The adjuvant effect of tubercle bacilli is due to a water-soluble peptide MDP (Muramyl Dipeptide) that induces good antibody response without causing granuloma. Given in mineral oil or as liposomes, it also stimulates cell-mediated immunity. Derivatives of MDP are being developed for human use.

Gram-negative bacilli as an adjuvant

These show adjuvant effect due to their lipopolysaccharide fraction. *Bordetella pertussis* – which has, in addition, lymphocytosis-promoting factor acting on both T and B cells – acts as a good adjuvant for diphtheria and tetanus toxoids in triple vaccine (DPT).

Vaccines

History

During the 15th century, an early form of smallpox vaccination was practiced in China and other parts of the world. Healthy people were intentionally infected with substances from the pustules of people suffering from smallpox, **a technique called variolation**. A mild form of smallpox usually resulted from this practice.

An English doctor, **Edward Jenner**, improved the variolation technique to create the first vaccine in **1796. Dr. Jenner** had heard that dairymaids who had been infected with cowpox, a disease resulted to but milder than smallpox, were not susceptible to smallpox, and decided to test the idea. He performed the first vaccination on a boy with material taken from lesions of cowpox. In fact, **the word vaccination comes from the Latin word for cow, vacca.**

The term "vaccine" was coined by Louis Pasteur to commemorate first successful immunization against small pox by Edward Jenner. The term vaccine was derived from "vacca", meaning cow, since Edward Jenner used cowpox virus (Vaccinia) to prevent smallpox infection.

Definition of vaccine

A vaccine is a suspension of whole (live or inactivated) or fractionated bacteria or viruses that have been rendered nonpathogenic, and is given to induce an immune response and prevent disease.

Methods of administration

The most common method of administering vaccine is by injections but some are given by mouth or by nasal spray.

Mechanism of action

Vaccine improves immunity to particular disease. Vaccine stimulates the body's immune system to recognize the agent as foreign, destroy it and remember it so that the immune system can more easily recognize and destroy these microorganisms when they enter in the vaccinated body.

Vaccine vial

The vaccine vial may contain relevant antigen, adjuvant (usually alum), preservatives and/or traces of protein derived from the cells in which the vaccine agent was cultured e.g. egg protein.

Properties of ideal vaccine:

- Provide long lasting immunity.
- Should induce both humoral and cellular immunity.
- Should not induce autoimmunity or hypersensitivity.
- Should be inexpensive to produce, easy to store and administer.
- Vaccines must also be perceived to be safe

Types of vaccines:

A. KILLED VACCINES
B. LIVE ATTENUATED VACCINE
C. SUBUNIT VACCINES
D. CONJUGATE VACCINES
E. RECOMBINANT VACCINES
F. DNA VACCINES

A. KILLED VACCINES

When it is unsafe to use live microorganisms to prepare vaccines, they are killed or inactivated. These are preparations of the normal (wild type)

infectious, pathogenic microorganisms that have been rendered non-pathogenic, usually by treatment with using heat, formaldehyde or gamma irradiation so that they cannot replicate at all. Such killed vaccines vary greatly in their efficacy.

Disease	Microorganism	Vaccine	Method	Route
Bacterial killed vaccines				
Typhoid	*Salmonella typhi, S. paratyphi A, S. paratyphi B*	TAB	Heat, Phenol, Acetone	SC (Sub-Cutaneous)
Cholera	*Vibrio cholerae*	Cholera	Phenol	SC or ID (Intra Dermal)
Plague	*Yersinia pestis*	Haffkin	Formalin	SC
Whooping Cough	*Bordetella pertussis*		Merthiolate	IM (Intra Muscular)
Viral Killed Vaccines				
Poliomyletis	PolioVirus	Salk	Formalin	IM
Rabies	Rabies virus	Semple	Phenol	SC
		BPL	BPL (Beta Propiolactone)	SC
		HDCV	BPL	IM or SC
		DEV	BPL	IM or SC
Flu	Influenza virus	-	Formalin	IM
Hepatitis	Hepatitis A	HM175	Formalin	IM

Advantages:

- ❖ Safe to use and can be given to immunodeficient and pregnant individuals.
- ❖ Cheaper than live attenuated vaccine
- ❖ Storage not as critical as live vaccine

Disadvantages:

- ❖ Since the microorganisms cannot multiply, a large number are required to stimulate immunity.

- ❖ Periodic boosters must be given to maintain immunity.
- ❖ Only humoral immunity can be induced.
- ❖ Most killed vaccines have to be injected.
- ❖ Some vaccines such as Bordetella pertussis induce ill effects like postvaccinial encephalomyelitis.
- ❖ Anaphylactic reaction may occur in (Inactivated Polio Vaccine) recipients.
- ❖ Anaphylactic hypersensitivity to eggs may occur in recipients of influenza vaccine.
- ❖ Inactivation, such as by formaldehyde in the case of the Salk vaccine, may alter antigenicity.
- ❖ Presence of some un-inactivated microbes can lead to vaccine-associated disease.

B. LIVE ATTENUATED VACCINE:

These vaccines are composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease.

To make an attenuated vaccine, the pathogen is grown in foreign host such as animals, embryonated eggs or tissue culture, under conditions that make it less virulent.

The strains are altered to a non-pathogenic form; for example, its tropism has been altered so that it no longer grows at a site that can cause disease.

Some mutants will be selected that have a better ability to grow in the foreign host. These tend to be less virulent for the original host.

These vaccines may be given by injection or by the oral route. A major advantage of live vaccines is that they cause infection but not disease hence booster doses are not required. The vaccine very closely reproduces the natural stimulus to the immune system.

Advantages:

- ❖ Infectious microbes can stimulate generation of memory cellular as well as humoral immune responses.
- ❖ Since these can multiply in the host, fewer quantities must be injected to induce protection.
- ❖ A single administration of vaccine often has a high efficacy in producing long-lived immunity. Multiple booster doses may not be required.

- ❖ Whole microbes stimulate response to antigens in their natural conformation. They raise immune response to all protective antigens.
- ❖ Some live vaccines can be given orally; such vaccines induce mucosal immunity and IgA synthesis, which gives more protection at the normal site of entry.
- ❖ Oral preparations are less expensive than giving injections.
- ❖ They can lead to elimination of wild type virus from the community.

Disease	Microorganism	Vaccine	Method	Route
Bacterial Live attenuated Vaccines				
Cholera	Vibrio	CVD103Hgr	Genetically Modified	Oral
Typhoid	Salmonella	Ty21a	Genetically Modified	Oral
TB	Mycobacterium	BCG	Prolonged subculture	ID (Intra Dermal)
Viral Live Attenuated Vaccines				
Poliomyletis	PolioVirus	Sabin	Passage in MK cells (Monkey Kidney)	Oral
Yellow Fever	Yellow Fever Virus	17 D	Passage in chick embryo cells	SC (Sub Cutaneous)
Flu	Influenza		Temperature sensitive mutant	IN (Intra Nasal)
Mesales, Mumps, Rubella	Mesales, Mumps, Rubella	MMR Rubella (Wistar RA 27/3)	Passage in fibroblasts cells	SC
Chicken pox	Chicken pox	Oka/Merck	Human diploid cell cultures	SC
Small pox	Small pox	Vaccinia virus	Naturally avirulent	ID

The influenza vaccine contains cold-adapted vaccine strain s of the influenza virus that has been grown in tissue culture at progressively lower temperatures. After a dozen or more of these passages, the virus grows well only at around 25° C and in vivo growth is restricted to the upper respiratory tract.

Disadvantages:

- ❖ May very rarely revert to its virulent form and cause disease.
- ❖ Live vaccines cannot be given safely to immunosuppressed individuals.
- ❖ Administration of live attenuated vaccines to people with impaired immune function can cause serious illness or death in the vaccine recipient.
- ❖ Since they are live and because their activity depends on their viability, proper storage is critical.

C. SUBUNIT VACCINES:

Subunit vaccines contain purified antigens instead of whole organisms. Such a preparation consists of only those antigens that elicit protective immunity. Subunit vaccines are composed of toxoids, subcellular fragments, or surface antigens. Administration of whole organism, as in case of pertussis was found unfavorable immune reactions resulting in severe side effects. The effectiveness of subunit vaccines is increased by giving them in adjuvants. Adjuvants slow antigen release for a more sustained immune stimulation.

Advantages:

- ❖ They can safely be given to immunosuppressed people
- ❖ They are less likely to induce side effects.

Disadvantages:

- ❖ Antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface.

- ❖ Isolated protein does not stimulate the immune system as well as a whole organism vaccine.

Disease	Microorganism	Vaccine	Subunit (Antigen)	Route
Bacterial Subunit vaccines				
Typhoid	*Salmonella typhi, S. paratyphi A, S. paratyphi B*	Vi (Typhim)	Cell Wall Polysaccharide	IM (Intra Muscular)
Bacterial Flu	*Hemophilus influenzae b*	Hib	Cell Wall Polysaccharide	IM
Meningitis	*Nesseria meningitides*	ACW-135Y	Cell Wall Polysaccharide	IM
pneumonia	*Streptococcus pneumoniae*	23 Valent	Cell Wall Polysaccharide	IM
Fever	*Group B Streptococcus*	-	Cell Wall Polysaccharide	IM
Tetanus (Dhanurwat)	*Clostridium tetani*	Tetanus Toxoid (TT)	Toxin	IM
Diptheria (Ghatsarp)	*Corynebacterium diphtheriae*	Diptheria Toxoid (DT)	Toxin	IM
Pertussis (Dangya Khokla)	*Bordetella pertussis*	Acelluar DTP	Microbial proteins	IM
Viral Subunit Vaccines				
Viral Flu	Influenza virus	-	Viral proteins	IM
Hepatitis	Hepatitis B	Hbs Ag	Viral proteins	IM
Protozoal Subunit Vaccines				
Malaria	Plasmodium vivax	spf66	Peptide	IM

D. CONJUGATE VACCINES:

Conjugate vaccines are primarily developed against capsulated bacteria. While the purified capsular antigen can act as subunit vaccine, they stimulate only humoral immunity. Polysaccharide antigens are T independent, they generate short-lived immunity. Immunity to these organisms requires opsonizing antibodies. Infants cannot mount good T-independent responses to polysaccharide antigens. By covalently linking the polysaccharides to protein carriers, they are converted into T-dependent antigens and protective immunity is induced.

Examples:

- ❖ Haemophilus influenza HiB polysaccharide is complexed with diphtheria toxoid.
- ❖ DPT vaccine, which combines Diphtheria toxoids, whole-cell Bordetella pertussis and Tetanus Toxoid.
- ❖ Tetraimmune vaccine, which combines the tetanus and diphtheria toxoids, whole-cell pertussis vaccine, and H. influenza type b conjugate vaccine.

E. RECOMBINANT VACCINES:

The vaccines are produced using recombinant DNA technology or genetic engineering. Recombinant vaccines are those in which genes for desired antigens of a microbe are inserted into a vector.

Different strategies are:

- ❖ Using the engineered vector (e.g., Vaccinia virus) that is expressing desired antigen as a vaccine.
- ❖ The engineered vector (e.g., yeast) is made to express the antigen, such is vector is grown and the antigen is purified and injected as a subunit vaccine. Other expression vectors include the bacteria Escherichia coli, mutant Salmonella spp., and BCG.
- ❖ Introduction of a mutation by deleting a portion of DNA such that they are unlikely to revert can create an attenuated live vaccine.
- ❖ Live attenuated vaccines can also be produced by re-assortment of genomes of virulent and avirulent strains.

- ❖ Genes coding for significant antigens are introduced into plants, such that the fruits produced bear foreign antigens. This is edible vaccine and is still in experimental stage.

Examples:

i. Hepatitis B Virus (HBV) vaccine is a recombinant subunit vaccine. Hepatitis B surface antigen is produced from a gene transfected into yeast (*Saccharomyces cerevisiae*) cells and purified for injection.
ii. Vaccinia virus may be engineered to express protein antigens of HIV, rabies etc. Foreign genes cloned into the viral genome are expressed on the surface of infected cells in association with class I MHC molecules. The antigen-MHC complex induces a Tc cell response.
iii. B subunit of cholera toxin, the B subunit of heat-labile *E. coli* enterotoxin (LT), and one of the glycoprotein membrane antigens of the malarial parasite are being developed using this technique.
iv. *Salmonella typhimurium* engineered to express antigens of *Vibrio cholera*
v. Bacille Calmette-Guérin vaccine strain engineered to express genes of HIV-1.
vi. Reassortment of genomes between human and avian strains to create Influenza vaccine. Human and swine strains to create Rotavirus vaccine.

Advantages:

- ❖ Those vectors that are not only safe but also easy to grow and store can be chosen.
- ❖ Antigens which do not elicit protective immunity or which elicit damaging responses can be eliminated from the vaccine. Example Cholera toxin A can be safely removed from cholera toxin.

Disadvantages:

- ❖ Since the genes for the desired antigens must be located, cloned, and expressed efficiently in the new vector, the cost of production is high.
- ❖ When engineered vaccinia virus is used to vaccinate, care must be taken to spare immunodeficient individuals.

F. DNA VACCINES:

These vaccines are still in experimental stage. Like recombinant vaccines, genes for the desired antigens are located and cloned. The DNA is injected into the muscle of the animal being vaccinated, usually with a "gene gun" that uses compressed gas to blow the DNA into the muscle cells. DNA can be introduced into tissues by bombarding the skin with DNA-coated gold particles. It is also possible to introduce DNA into nasal tissue in nose drops. Some muscle cells express the pathogen DNA to stimulate the immune system. DNA vaccines have induced both humoral and cellular immunity.

Advantages:

- DNA is very stable, it resists extreme temperature and hence storage and transport are easy.
- A DNA sequence can be changed easily in the laboratory.
- The inserted DNA does not replicate and encodes only the proteins of interest. There is no protein component and so there will be no immune response against the vector itself.
- Because of the way the antigen is presented, there is a cell-mediated response that may be directed against any antigen in the pathogen.

Disadvantages:

- Potential integration of DNA into host genome leading to insertional mutagenesis.
- Induction of autoimmune responses: anti-DNA antibodies may be produced against introduced DNA.
- Induction of immunologic tolerance: The expression of the antigen in the host may lead to specific non-responsiveness to that antigen.

Principles, methods of production and administration of BCG, TAB, OPV, T.T., DPT vaccines

BCG Vaccine

BCG or Bacille Calmette Guerin is a vaccine against Tuberculosis disease (TB). BCG (Bacillus of Calmette Guerin) is named after the two Frenchmen who developed it.

BCG vaccine is an attenuated, live culture preparation of the Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis Danish strain 1331 suspended in monosodium glutamate.

Vaccination with BCG Vaccine elicits a cell mediated immune response that con fers a variable degree of protection to infection with M tuberculosis.

Preparation

Mycobacterium bovis Danish strain 1331 is attenuated by successive subculturing 239 times in Glycerol Bile Potato medium for over 13 years. Vaccine strains are prepared by growing the bacilli in an artificial medium, harvesting, concentrating, homogenizing, and the preparation is then lyophilized.

The medium in which the BCG organism is grown for preparation of the freeze-dried is composed of the following ingredients:

- Glycerin,
- Asparagine,
- Citric acid,
- Potassium phosphate,
- Magnesium sulfate,
- Iron ammonium citrate.

The final preparation prior to freeze drying also contains lactose. The freeze-dried BCG preparation is delivered in vials, each containing 1 to 8 x 10^8 colony forming units (CFU) of BCG which is equivalent to approximately 50 mg wet weight

Dose

Adults and Children aged 12 months and over

After reconstitution, 1 dose (0.1 ml) for contains:
Mycobacterium bovis BCG (Bacillus Calmette-Guerin), , live attenuated, 2-8 x 10^5 cfu.

For infants under 12 months of age

After reconstitution, 1 dose (0.05 ml) contains:
Mycobacterium bovis BCG (Bacillus Calmette-Guerin), live attenuated, 1-4 x 10^5 cfu.

Method of administration

BCG is given as a single intradermal injection at the insertion of the deltoid. If BCG is accidentally given subcutaneously, then a local abscess may form (a "BCG-oma") that can sometimes ulcerate, and may require treatment with antibiotics immediately, otherwise without treatment it could spread the infection causing severe damage to vital organs.

Medical uses

1) Tuberculosis: The main use of BCG is for vaccination against tuberculosis. BCG vaccine can be implemented after the birth intradermally.
2) Leprosy: BCG has a protective effect against leprosy in the range of 26 to 41% based on controlled trials.
3) Buruli ulcer: BCG may protect against or delay the onset of Buruli ulcer.
4) Cancer immunotherapy/cancer vaccine: A number of cancer vaccines use BCG as an adjuvant to provide an initial stimulation of the patients' immune systems. BCG is used in the treatment of superficial forms of bladder cancer, Colorectal cancer, Lung cancer, Melanoma.
5) Diabetes, type 1: Clinical trials based on the work of Denise Faustman use BCG to induce production of TNF-α, which can kill the T-cells responsible for type 1 diabetes. In a Phase I, double-blind, placebo-controlled trial, two doses of the BCG vaccine were administered to three

adults with long-term type 1 diabetes, resulting in elimination of the pancreas-harming cells, induction of regulatory T cells (Tregs) and a transient rise in C-peptide levels, suggestive of temporarily restored insulin production.

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TAB Vaccine

TYPHOID VACCINES

Typhoid fever is caused by the enteric bacterium *Salmonella typhi, S. paratyphi* A and *S.paratyphi* B. Active immunization against typhoid fever has been achieved with some success by vaccines. The various vaccines that have been employed over the years to prevent typhoid fever can be divided into five broad groups.

1. Inactivated whole cell parenteral vaccine

2. Subunit parenteral vaccine

3. Inactivated whole cell oral vaccine

4. Polysaccharide -carrier protein conjugate parenteral vaccine

5. Live attenuated oral vaccine

Inactivated whole cell parenteral vaccines:

1. Heat inactivated phenol preserved vaccine (TAB vaccine):

TAB vaccine is against typhoid.

Preparation

TAB vaccine Prepared by heating *S.typhi, S.paratyphi* A and *S.paratyphi* B cultures at 56^{o}C for one hour and then suspending in 0.5% phenol solution.

This vaccine contains -

- 1000 million bacteria per ml of S.typhi
- 750 million bacteria per ml of *S.paratyphi* A
- 750 million bacteria per ml of *S.paratyphi* B.

Dose

Primary immunization with this parenteral vaccine consists of two doses given four weeks apart with a single booster dose recommended every three years. 0.5 ml of vaccine is given in two subcutaneous doses at an interval of 4-6 weeks, which is followed by intradermal injection of 0.1 ml every three years. Subcutaneous injections can result in local reactions, which can be avoided by injecting 0.1ml intradermally. Certain countries use vaccines containing only inactivated *S.typhi* or a divalent vaccine consisting of *S.typhi* and *S.paratyphi* A cultures. In endemic areas vaccination is recommended for all children, in whom single dose might give sufficient protection, which may be maintained for several years by booster doses. This vaccine induces only humoral immunity but no local or cellular immunity.

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OVP Vaccine (Oral Polio Vaccine)

OPV vaccine is against Poliomyletis (Polio) disease.

Two **polio vaccines** are used throughout the world to combat poliomyelitis (or polio).

Salk dead polio virus vaccine: The first was developed by Jonas Salk through the use of HeLa cells and first tested in 1952. It consists of an injected dose of inactivated (dead) poliovirus.

Sabin attenuated polio virus / Oral Polio Vaccine (OPV) An oral vaccine was developed by Albert Sabin using attenuated poliovirus. Human trials of Sabin's vaccine began in 1957, and it was licensed in 1962.

The two vaccines have eradicated polio from most countries in the world, and reduced the worldwide incidence from an estimated 350,000 cases in 1988 to just 223 cases in 2012.

Oral polio vaccine (OPV)

The oral polio vaccine (OPV) was developed in 1961 by Albert Sabin. Also called “trivalent oral polio vaccine” or “Sabin vaccine”, OPV consists of a mixture of live, attenuated (weakened) poliovirus strains of all three poliovirus types.

Oral polio vaccines were developed by several groups, one of which was led by Albert Sabin. Other groups, developed their own attenuated vaccine strains. In 1958, the National Institutes of Health created a special committee on live polio vaccines who the Sabin strains for worldwide distribution.

Production

There are 3 strains of Polio virus used by Sabin for attenuation. It is a live-attenuated vaccine, produced by the passage of the virus through non-human cells at a sub-physiological temperature, which produces spontaneous mutations in the viral genome.

Sabin 1 strain

There are 57 nucleotide substitutions which distinguish the attenuated Sabin 1 strain from its virulent parent (the Mahoney serotype),

Sabin 2 strain

Two nucleotide substitutions attenuate the Sabin 2 strain,

Sabin 3 strain

10 substitutions are involved in attenuating the Sabin 3 strain.

The primary attenuating factor common to all three Sabin vaccines is a mutation located in the virus's internal ribosome entry site (IRES) which alters stem-loop structures, and reduces the ability of poliovirus to translate its RNA template within the host cell.

Mechanism

The attenuated poliovirus in the Sabin vaccine replicates very efficiently in the gut, the primary site of infection and replication, but is unable to replicate

efficiently within nervous system tissue. OPV produces antibodies in the blood to all three types of poliovirus. In the event of infection, these antibodies protect against paralysis by preventing the spread of wild poliovirus to the nervous system. OPV also produces a local, mucosal immune response in the mucous membrane of the intestines. In the event of infection, these mucosal antibodies limit the replication of the wild poliovirus inside the intestine. This intestinal immune response to OPV is thought to be the main reason why mass campaigns with OPV can rapidly stop person-to-person transmission of wild poliovirus.

Advantages

- OPV is administered orally. It can be given by volunteers and does not require trained health workers or sterile injection equipment.
- The vaccine is relatively inexpensive.
- OPV is safe, effective, and induces long-lasting immunity to all three types of poliovirus.
- For several weeks after vaccination, the vaccine virus replicates in the intestine, is excreted in the faeces, and can be spread to others in close contact. This means that in areas where hygiene and sanitation are poor, immunization with OPV can result in the "passive" immunization of people who have not been directly vaccinated.

Disadvantages

- Although OPV is safe and effective, in extremely rare cases (approx. 1 in every 2.7 million first doses of the vaccine) the live attenuated vaccine virus in OPV can cause paralysis. In some cases it is believed that this vaccine-associated paralytic polio (VAPP) may be triggered by immune deficiency.
- A second disadvantage is that very rarely the virus in the vaccine may genetically change and start to circulate among a population. These viruses are known as circulating vaccine-derived polioviruses (cVDPV).

Dose

One dose of OPV produces immunity to all three poliovirus serotypes in approximately 50% of recipients. Three doses produce immunity in more than 95% of recipients. Immunity is long-lasting and probably life-long.

OPV is usually provided in vials containing 10-20 doses of vaccine. A single dose of oral polio vaccine (usually two drops) contains

- 1,000,000 infectious units of Sabin 1 (effective against PV1),
- 100,000 infectious units of the Sabin 2 strain,
- 600,000 infectious units of Sabin 3.

The vaccine contains small traces of antibiotics— neomycin and streptomycin—but does not contain preservatives. One dose of OPV produces immunity to all three poliovirus serotypes in approximately 50% of recipients. Three doses of live-attenuated OPV produce protective antibody to all three poliovirus types in more than 95% of recipients.

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TT (Tetanus Toxoid)

Tetanus Toxoid is used to prevent tetanus (also known as lockjaw). Tetanus is a serious illness caused by toxin produced by *Clostridium tetani* that causes convulsions (seizures) and severe muscle spasms that can be strong enough to cause bone fractures of the spine. Tetanus causes death in 30 to 40 percent of cases.

Tetanus Toxoid, for intramuscular or subcutaneous use, is a sterile solution of toxoid in isotonic sodium chloride solution. The vaccine is clear or slightly turbid in appearance

Preparation

- Clostridium tetani culture is grown in a peptone-based medium and detoxified with formaldehyde.
- The detoxified material is then purified by serial ammonium sulfate fractionation, followed by sterile filtration.

- The toxoid is then diluted with physiological saline solution (0.85%). Each dose contains the preservative thimerosal [(mercury derivative), 25 µg mercury/dose].
- This product does not contain an aluminum-containing adjuvant.
- 0.1 to 1.0% formaldehyde, in the form of formalin, was added to an aliquot and the mixture was pre incubated for 30 minutes at 37° C. L-lysine monohydrochloride was subsequently added to a final concentration of 0.005 to 0.1M, in the presence of 0.1M sodium bicarbonate. The pH of the reaction mixture was from 6.5 to 7.5 and toxoiding was allowed toproceed for 14 days at 37° C .
- Each 0.5 mL dose is formulated to contain 4 Lf (flocculation units) of tetanus toxoid and passes the guinea pig potency test. The residual formaldehyde content, by assay, is less than 0.02%.

Dose

Primary immunization schedule for children under 7 years of age (prior to seventh birthday) should consist of five doses of a vaccine containing tetanus toxoid. The initial three doses are given as Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) vaccine, administered intramuscularly at intervals of 4 to 8 weeks. A fourth dose of DTaP is recommended at 15 to 20 months of age. The interval between the third and fourth dose should be at least 6 months. A fifth dose of DTaP is given before school entry (kindergarten or elementary school) at 4 to 6 years of age.

The Tetanus Toxoid (TT) vaccine is given during your pregnancy to prevent tetanus to you as well as your baby. In the first pregnancy, your doctor will recommend at least two doses of the TT vaccine. The first vaccination is given in the first trimester soon after your pregnancy tests are confirmed and after your first antenatal appointment. The second dose of the TT vaccine is given at least four to eight weeks after the first. Some experts recommend that the second dose of the vaccine should be given four weeks prior to the expected date of delivery. The WHO also recommends that a third vaccine be given six months after the second one to provide protection for at least five years.

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DTP VACCINE

DTP is a trivalent vaccine containing diphtheria toxoid, tetanus toxoid and pertussis vaccine (Whole Cell Pertussis). It is also referred to as triple antigen. This vaccine is aimed at inducing active immunity in children against diphtheria, tetanus and whooping cough (pertussis).

It consists of toxoids of diphtheria and tetanus along with killed whole cell suspension of *Bordetella pertussis*. Each of the components can be given separately, but administering them in combination minimizes the number of injections and improves the immune response against toxoids because the pertussis bacilli act as an adjuvant.

Vaccine preparation and composition:

Each 0.5-ml dose is formulated to contains-

- 6.7-12.5 Lf units of diphtheria toxoid
- 5 Lf units of tetanus toxoid,
- less than or equal to 16 opacity units of pertussis vaccine.

The cells of *Bordetella pertussis* in DTP vaccine are inactivated either with formalin or treatment with 0.2% merthiolate at 4^{o}C for several months. Toxins are toxoided either by heating or treatment with formaldehyde.

Course of vaccine:

Primary course of DPT immunization consists of three intramuscular injections at intervals of 4-6 weeks before the age of six months. The first dose is usually started by 2 months of age. This is followed by a booster dose at the end of first year of life. Subsequent booster doses are given at five years of age and 10 years of age. Even though the infant receives maternal antibodies against tetanus, the immunization is started by 2 months because there is very little maternal immunity against pertussis. Diphtheria and pertussis are uncommon after the age of 5 years but tetanus can occur at any age. Therefore a booster dose of tetanus toxoid is administered at school entry.

Side effects of vaccine:

The side effects, whenever seen are usually because of pertussis component in the vaccine. About 20% of the children, who receive the whole cell vaccine experience mild side effects such as local inflammation and fever. About 0.1% of infants experience convulsions soon after receiving the vaccine and in a very small number of cases (1 in 150,000) severe or irreversible brain damage may occur.

Immunization Schedule

The schedule recommended by the Government of India has fully incorporated the recommendations of the EPI schedule given by the World Health Organisation.

The Government of India recommends the following schedule for vaccinations:

Age	Vaccine
Birth	BCG, OPV Dose 1, Hep B (Hepatitis B vaccine) Dose 1
6 – 8 weeks	DPT Dose1, HIB (Haemophilus Influenzae type B vaccine) Dose 1, PCV (Pneumococcal Conjugate vaccine) Dose 1, Rotavirus Dose 1, OPV Dose 2 , Hep B Dose 2
10 - 16 weeks	DPT Dose 2, HIB Dose 2, PCV Dose 2, Rotavirus Dose 2, OPV Dose 3.
14 – 24 weeks	DPT Dose 3, HIB Dose 3, PCV Dose 3, Rotavirus Dose 3, OPV Dose 4.
6 months	Influenza vaccine Dose 1
7- 8 months	Influenza vaccine Dose 2
9-12 months	Measles vaccine, OPV Dose 5.
12-18 months	Hep A (Hepatitis A vaccine) Dose 1, Chickenpox.
15 – 18 months	MMR (Measles, mumps and rubella vaccine), HIB Booster , DTP booster.
18-24 months	OPV Booster 1, Hep A Dose 2.
2 years	Typhoid vaccine, Meningococcal meningitis.
4-5 years	OPV Boster 2, Typhoid Dose 2, MMR Booster, DPT Booster 2, Chickenpox Booster.

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Immune Disorders

Like any system in a vertebrate host, disorders (malfunctions) also occur in the immune system. Immune disorders can be categorized as

- Hypersensitivities
- Autoimmune diseases
- Transplantation (tissue) rejection
- Immunodeficiencies.

Hypersensitivity

Hypersensitivity is an exaggerated immune response that results in tissue damage and is manifested in the individual on second or subsequent contact with an antigen. Hypersensitivity reactions can be classified as either immediate or delayed. Obviously immediate reactions appear faster than delayed ones, but the main difference between them is in the nature of the immune response to the antigen. The Gell-Coombs classification system divides hypersensitivity into four types:

- Type I Hypersensitivity (Allergy)
- Type II Hypersensitivity (Cytolytic or Cytotoxic reaction)
- Type III Hypersensitivity (Formation of Immune Complexes)
- Type IV Hypersensitivity (Delayed Type Hypersensitivity/ DTH)

Type I Hypersensitivity (Allergy):

An **allergy** [Greek *allos,* other and *ergon,*work] is one kind of type I hypersensitivity reaction. It is characterized by an allergic reaction occurring immediately following an individual's second contact with the responsible antigen (the allergen). Upon initial exposure to a soluble allergen, B cells are stimulated to differentiate into plasma cells and produce specific IgE with the help of T cells. This IgE is sometimes called a **reagin,** and the individual has a hereditary predisposition for its production. Once synthesized, IgE binds to the

Fc receptors of mast cells (basophils and eosinophils can also be activated) and sensitizes these cells, making the individual allergic to the allergen.

When a second exposure to the allergen occurs, the allergen attaches to the surface bound IgE on the sensitized mast cells causing degranulation. Degranulation releases physiological mediators such as histamine, leukotrienes, heparin, prostaglandins, PAF (platelet-activation factor), ECF-A (eosinophil chemotactic factor of anaphylaxis), and proteolytic enzymes.

These mediators trigger smooth muscle contractions, vasodilation, increased vascular permeability, and mucous secretion. The inclusive term for these responses is **anaphylaxis.**

Anaphylaxis can be divided into systemic and localized reactions.

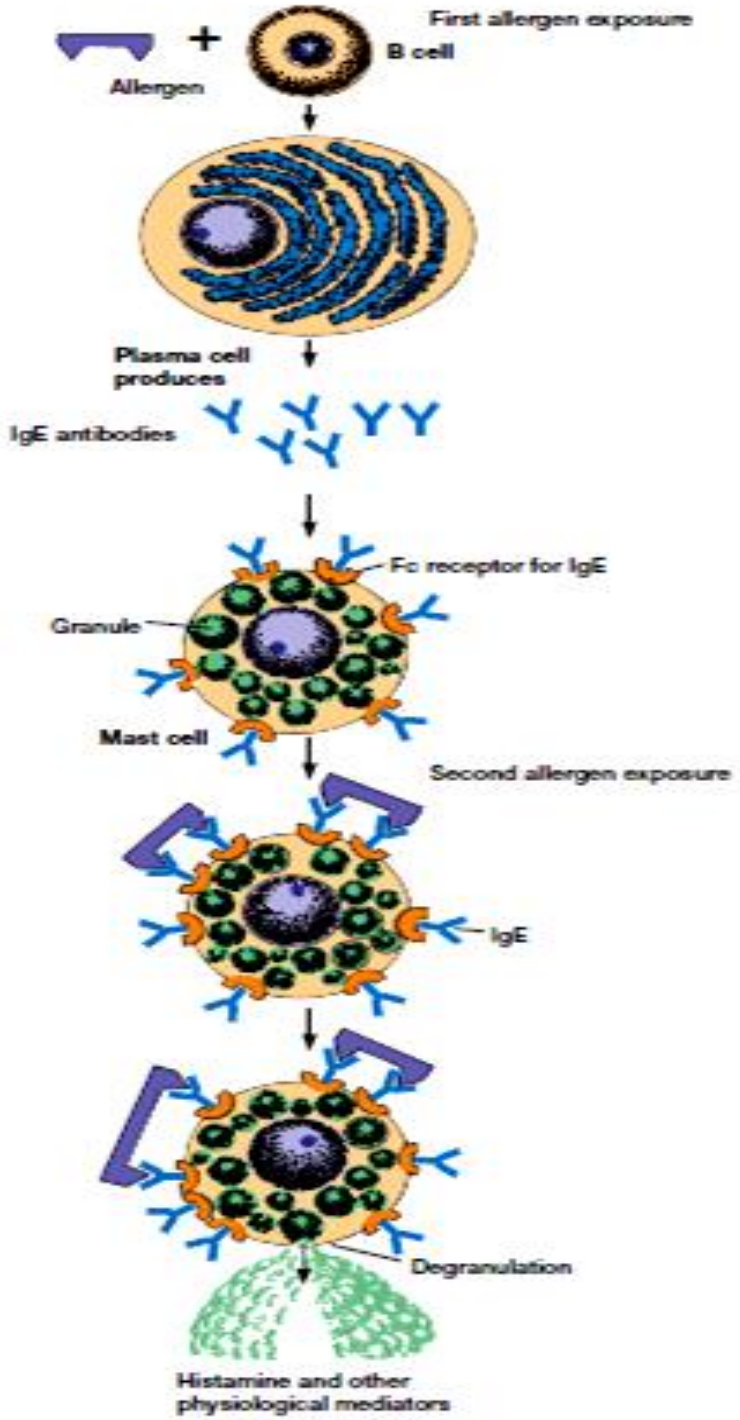
Systemic anaphylaxis

It is a generalized response that occurs when an individual sensitized to an allergen receives a subsequent exposure to it. The reaction is immediate due to the large amount of mast cell mediators released over a short period. Usually there is respiratory impairment caused by smooth muscle constriction in the bronchioles. The arterioles dilate, which greatly reduces arterial blood pressure and increases capillary permeability with rapid loss of fluid into the tissue spaces. Because of these reactions the individual can die within a few minutes from reduced venous return, asphyxiation, reduced blood pressure, and circulatory shock.

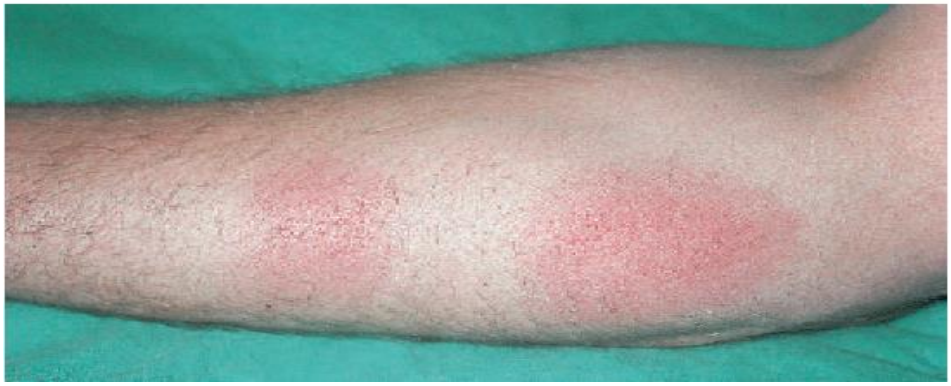
Common examples of allergens that can produce systemic anaphylaxis include drugs ***(penicillin), passively administered antisera, peanuts, and insect venom from the stings or bites of wasps, hornets, or bees.***

Localized anaphylaxis:

It is called an atopic ("out of place") allergy. The symptoms that develop depend primarily on the route by which the allergen enters the body. **Hay fever** (allergic rhinitis) is a good example of an atopic allergy involving the upper respiratory tract. Initial exposure involves airborne allergens—such as plant pollen, fungal spores, animal dander, and house dust mites—that sensitize mast cells located within the mucous membranes. Re-exposure to the allergen causes the typical localized anaphylactic response: itchy and tearing eyes, congested nasal passages, coughing, and sneezing. Antihistamine drugs are used to help alleviate these symptoms.



Type I Hypersensitivity. This type of hypersensitivity occurs when IgE antibodies attach to mast cells. The combination of these antibodies with allergens stimulates the mast cell (or basophil) to degranulate and produce the physiological mediators that cause the anaphylactic reaction, asthma, or hay fever.

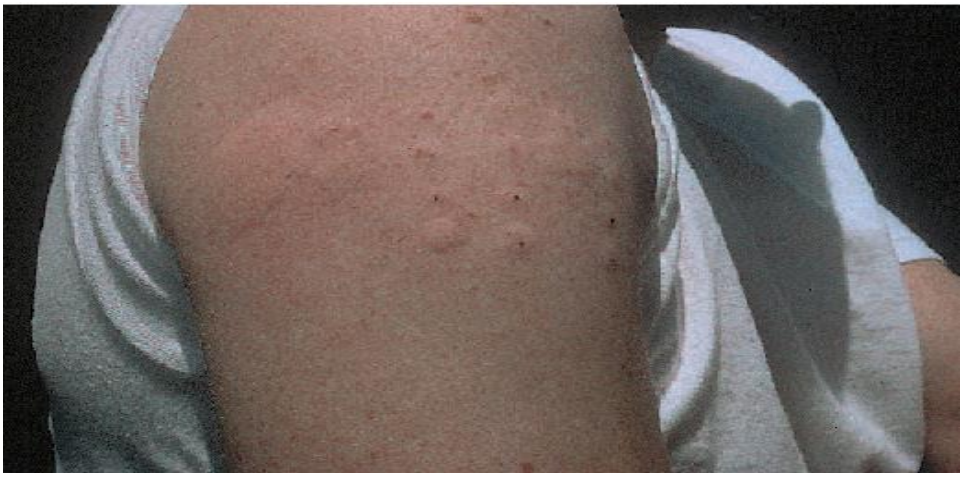


The Anaphylactic Response to Bee Venom. This person has been stung on the arm by a bee, leading to type I hypersensitivity in a generalized area.

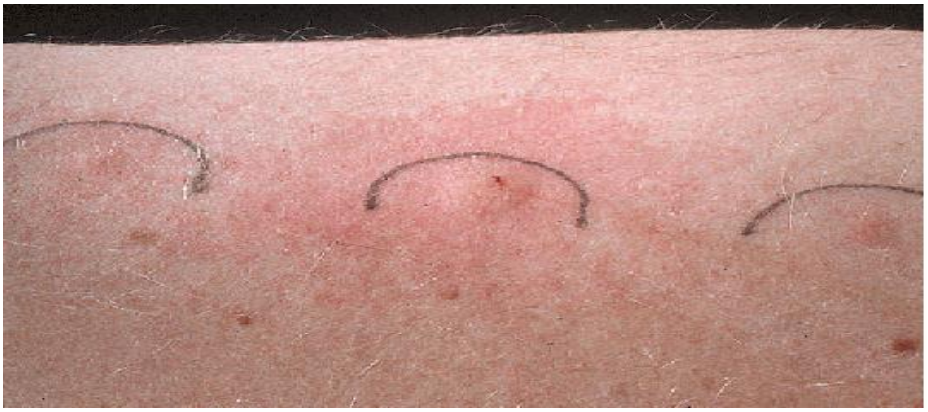
Bronchial asthma (asthma means panting) is an example of an atopic allergy involving the lower respiratory tract. Common allergens are the same as for hay fever. In bronchial asthma, however, the air sacs (alveoli) become over-distended and fill with fluid and mucus; the smooth muscle contracts and narrows the walls of the bronchi. Bronchial constriction produces a wheezing or whistling sound during exhalation. Symptomatic relief is obtained from bronchodilators that help relax the bronchial muscles, and from expectorants and liquefacients that dissolve and expel mucous plugs that accumulate.

Allergens that enter the body through the digestive system may cause food allergies. **Hives** (eruptions of the skin) are a good diagnostic sign of a true food allergy. Once established, type I food allergies are usually permanent but can be partially controlled with antihistamines or by avoidance of the allergen.

Skin testing can be used to identify the allergen responsible for allergies. These tests involve inoculating small amounts of suspect allergen(s) into the skin. Sensitivity to the antigen is shown by a rapid inflammatory reaction characterized by redness, swelling, and itching at the site of inoculation. The affected area in which the allergen-mast cell reaction takes place is called a wheal and flare reaction site.



(**a**) Skin prick tests with grass pollen in a person with summer hay fever. Notice the various reactions with increasing dosages (from top to bottom).

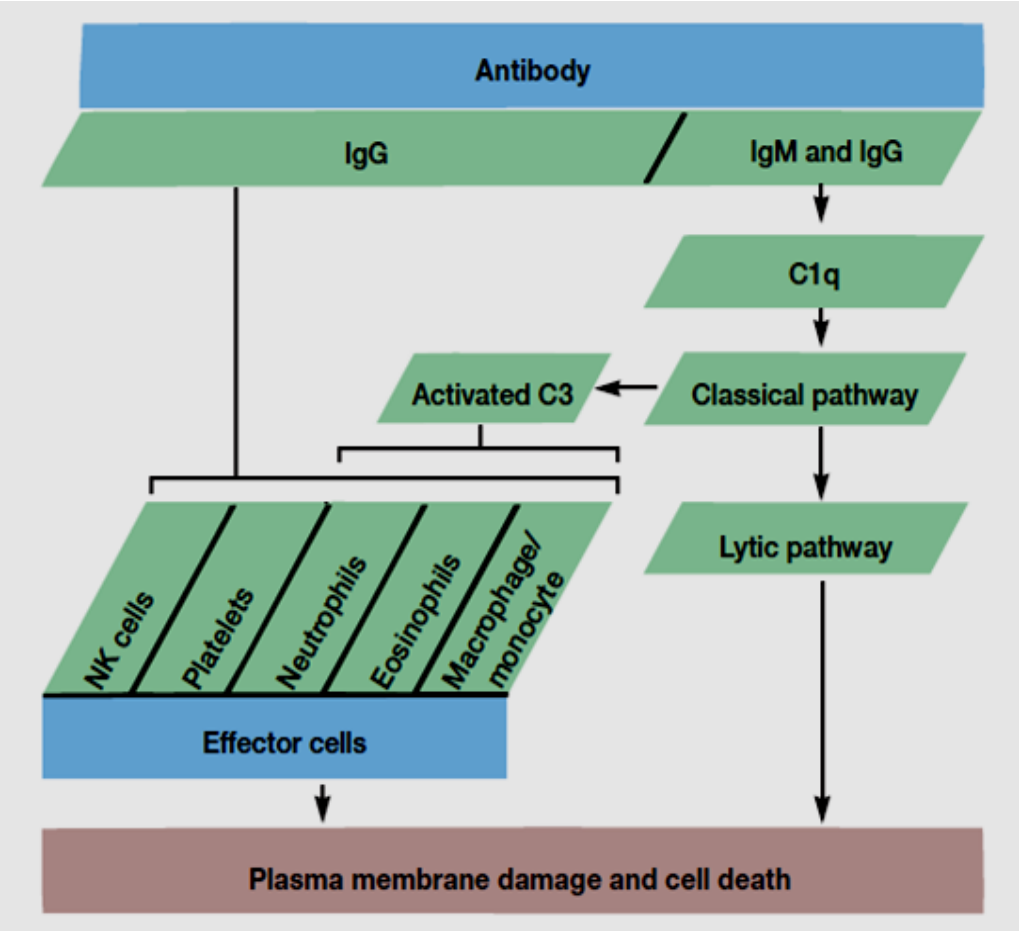


(**b**) Skin patch test. The surface of the skin (left) is abraded and the suspect allergic extract placed on the skin. After 48 hours (center) it is eczematous and positive for the suspect antigen.

Once the responsible allergen has been identified, the individual should avoid contact with it. At times this is not possible, and **desensitization** is warranted. This procedure consists of a series of allergen doses injected beneath the skin to stimulate the production of IgG antibodies rather than IgE antibodies. The circulating IgG antibodies can then act as blocking antibodies to intercept and neutralize allergens before they have time to react with mast cell-bound IgE. Recent evidence suggests that suppressor T-cell activity also may cause a decrease in IgE synthesis. Desensitizations are about 65 to 75% effective in individuals whose allergies are caused by inhaled allergens.

Type II Hypersensitivity (cytolytic or cytotoxic Reaction)

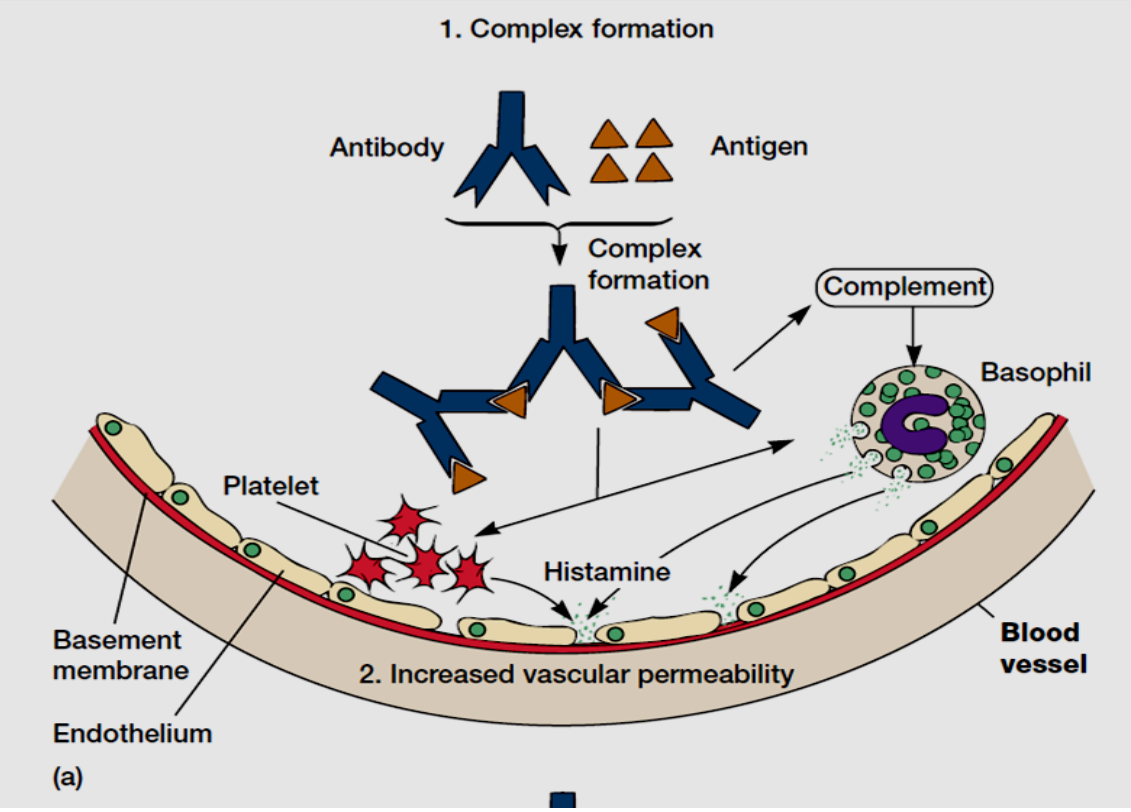
Type II hypersensitivity is generally called a cytolytic or cytotoxic reaction because it results in the destruction of host cells, either by lysis or toxic mediators. In type II hypersensitivity, IgG or IgM antibodies are directed against cell surface or tissue-associated antigens. They usually stimulate the complement pathway and a variety of effector cells. The antibodies interact with complement (Clq) and the effector cells through their Fc regions. The damage mechanisms are a reflection of the normal physiological processes involved in interaction of the immune system with pathogens. A classic example of type II hypersensitivity is that resulting when a person receives a transfusion with blood from a donor with a different blood group.



Type II Hypersensitivity. The action of antibody occurs through effector cells or the membrane attack complex, which damages target cell plasma membranes, causing cell destruction.

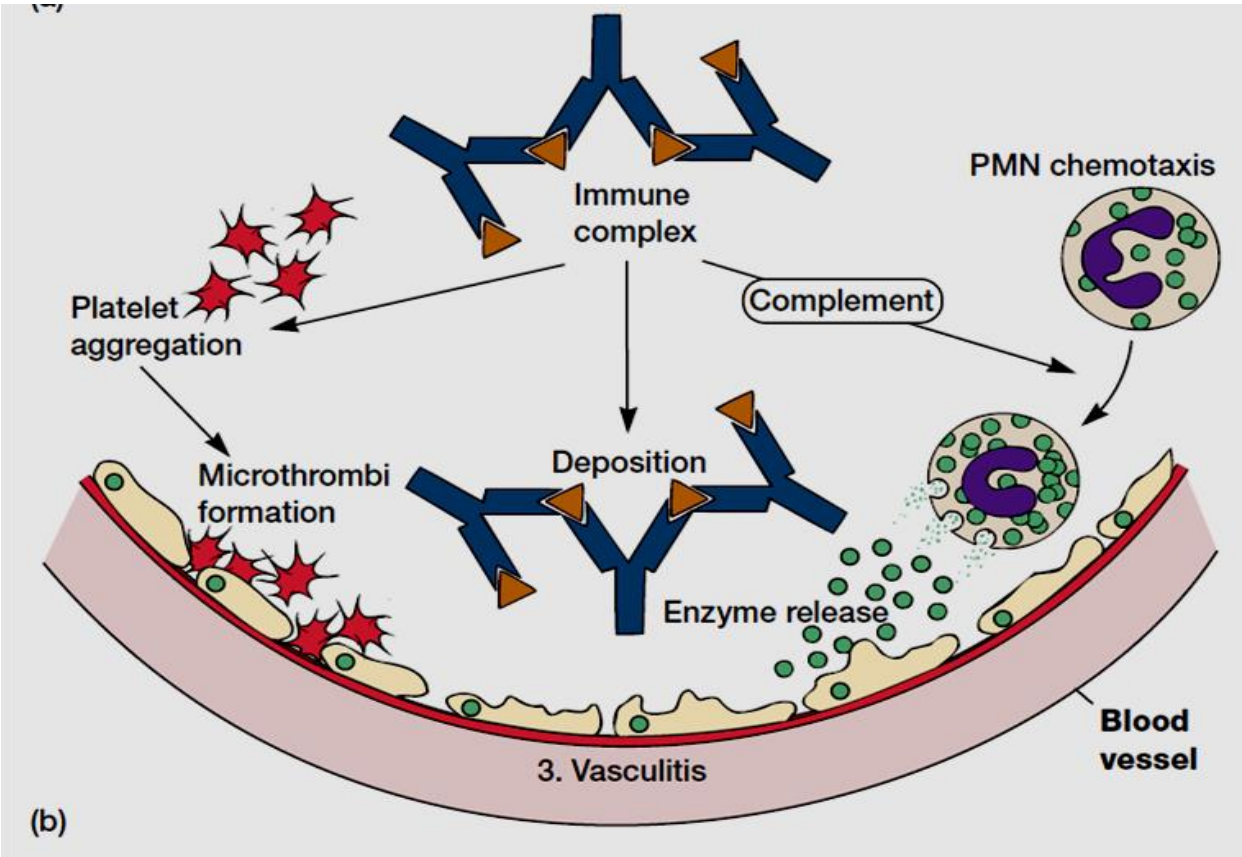
Type III Hypersensitivity (Formation of immune Complexes)

Type III hypersensitivity involves the formation of immune complexes. Normally these complexes are removed effectively by the fixed monocytes and macrophages of the monocyte-macrophage system. In the presence of excess amounts of some soluble antigens, the antigen-antibody complexes may not be efficiently removed. Their accumulation can lead to a hypersensitivity reaction from complement that triggers a variety of inflammatory processes.



Type III Hypersensitivity. Deposition of immune complexes in blood vessel walls. (**a**) Antibody and antigen combine to form immune complexes. These activate complement, which causes basophils and platelets to degranulate and release histamine and other mediators. These mediators increase vascular permeability.

This inflammation causes damage, especially of blood vessels (vasculitis), kidney glomerular basement membranes (glomerulonephritis), **joints (arthritis)**, and skin.



(**b**) The increased permeability allows the immune complexes to be deposited in the blood vessel wall. This induces platelet aggregation to form microthrombi (blood clots) on the vessel wall. PMNs, stimulated by complement, degranulate causing enzymatic damage to the blood vessel wall.

Diseases resulting from type III reactions can be placed into three groups.
i) A persistent viral, bacterial, or protozoan infection, together with a weak antibody response, leads to chronic immune complex formation and eventual deposition of the complex in host tissues.
ii) The continued production of autoantibody to self-antigen during an autoimmune disease can lead to prolonged immune complex formation. This overloads the monocyte-macrophage system, and tissue deposition of the complexes occurs (e.g., in the disease **systemic lupus erythematosus**).
iii) Immune complexes can form at body surfaces (such as the lungs), following repeated inhalation of allergens from molds, plants, or animals. For example, in Farmer's lung disease, an individual has circulating antibodies to fungi after being exposed repeatedly to moldy hay. These antibodies are primarily IgG. When the allergens (fungal spores) enter the alveoli of the lungs, local immune complexes form, leading to inflammation.

Type IV Hypersensitivity (Delayed Type Hypersensitivity DTH)

Type IV hypersensitivity involves delayed T-cell-mediated immune reactions. A major factor in the type IV reaction is the time required for a special subset of T_H1 cells (often called delayed type hypersensitivity [TDTH] cells) to migrate to and accumulate near the antigens. This usually takes a day or more.

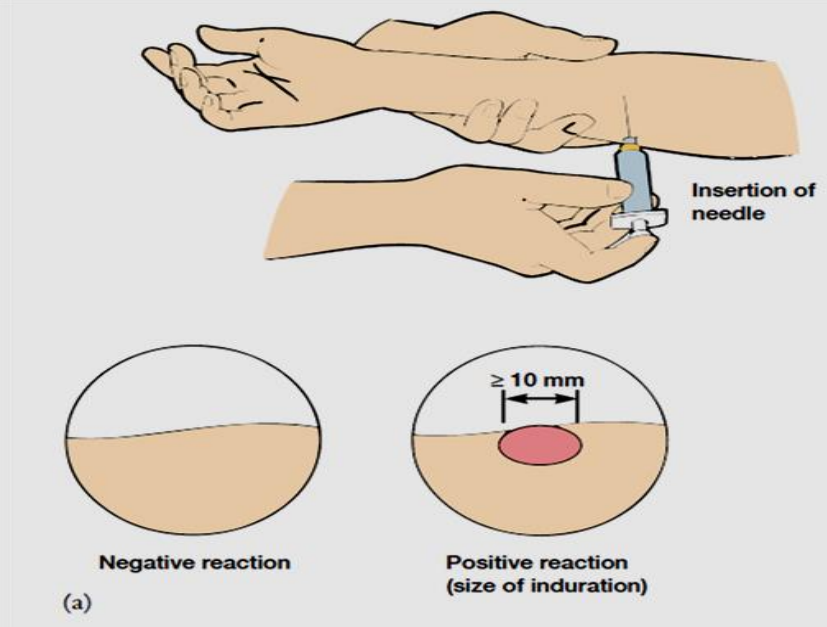
Type IV reactions occur when antigens, especially those binding to tissue cells, are phagocytosed by macrophages and then presented to receptors on the T_H1 cell surface in the context of class I MHC. Contact between the antigen and T_H1 cell causes the cell to proliferate and release cytokines. Cytokines attract lymphocytes, macrophages, and basophils to the affected tissue. Extensive tissue damage may result.

Examples of type IV hypersensitivities include -

- Tuberculin hypersensitivity (the **TB skin test**)
- Allergic contact dermatitis
- Some autoimmune diseases
- Transplantation rejection
- Killing of cancer cells.

In tuberculin hypersensitivity a partially purified protein called tuberculin, which is obtained from the bacillus that causes tuberculosis (*Mycobacterium tuberculosis*), is injected into the skin of the forearm. The response in a tuberculin-positive individual begins in about 8 hours, and a reddened area surrounding the injection site becomes indurated (firm and hard) within 12 to 24 hours. The T_H1 cells that migrated into the injection site are responsible for the induration. The reaction reaches its peak in 48 hours and then subsides. The size of the induration is directly related to the amount of antigen that was introduced and to the degree of hypersensitivity of the tested individual.

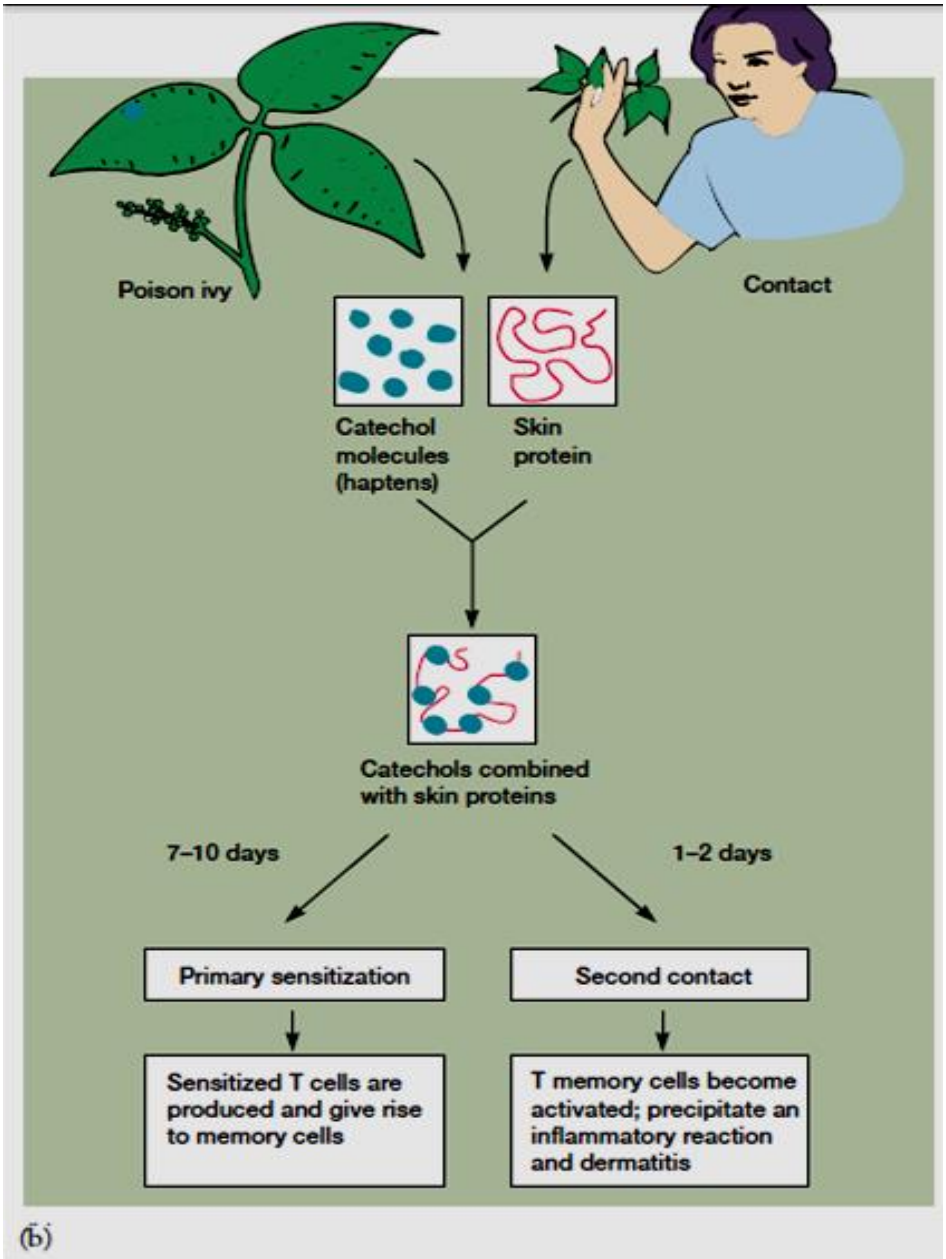
Other microbial products used in type IV skin testing are histoplasmin for histoplasmosis, coccidioidin for coccidioidomycosis, lepromin for leprosy, and brucellergen for brucellosis.



Mechanism of Type IV Hypersensitivity. (**a**) The tuberculin skin test. A positive reaction is one in which the diameter of induration is 10 mm or more.

Allergic contact dermatitis is caused by haptens that combine with proteins in the skin to form the allergen that elicits the immune response. The haptens are the antigenic determinants, and the skin proteins are the carrier molecules for the haptens. Examples of these haptens include cosmetics, plant materials (catechol molecules from poison ivy and poison oak), topical chemotherapeutic agents, metals, and **jewelry (especially jewelry containing nickel)**.

Several important chronic diseases involve cell and tissue destruction by type IV hypersensitivity reactions. These diseases are caused by viruses, mycobacteria, protozoa, and fungi that produce chronic infections in which the macrophages and T cells are continually stimulated. Examples are leprosy, tuberculosis, leishmaniasis, candidiasis, and herpes simplex lesions.



(**b**) In this example of contact dermatitis to poison ivy, a person initially becomes exposed to the catechol molecules from the poison ivy plant. The catechols combine with high molecular weight skin proteins and act as haptens. After 7–10 days sensitized T cells are produced and give rise to memory T cells. Upon second contact, the catechols bind to the same skin proteins, and the T memory cells become activated in only 1–2 days, leading to the inflammatory reaction (contact dermatitis).