

## Serology

**Serology** is the scientific study of blood serum. In practice, the term usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins (in response, for example, to a mismatched blood transfusion), or to one's own proteins (in instances of autoimmune disease). Serological tests may be performed for diagnostic purposes when an infection is suspected, in rheumatic illnesses, and in many other situations, such as checking an individual's blood type. Serology blood tests help to diagnose patients with certain immune deficiencies associated with the lack of antibodies, such as X-linked agammaglobulinemia. In such cases, tests for antibodies will be consistently negative.

There are several serology techniques that can be used depending on the antibodies being studied. These include: ELISA, agglutination, precipitation, complement-fixation, and fluorescent antibodies. Some serological tests are not limited to blood serum, but can also be performed on other bodily fluids such as semen and saliva, which have (roughly) similar properties to serum.

Serological tests may also be used forensically, generally to link a perpetrator to a piece of evidence (e.g., linking a rapist to a semen sample). Serology is a blood test to detect the presence of antibodies against a microorganism. Certain microorganisms stimulate the body to produce antibodies during an active infection.

### How the Test is Performed

Blood is drawn from a vein, usually from the inside of the elbow or the back of the hand. The site is cleaned with germ-killing medicine (antiseptic). The health care provider wraps an elastic band around the upper arm to apply pressure to the area and make the vein swell with blood. Next, the health care provider gently inserts a needle into the vein. The blood collects into an airtight vial or tube attached to the needle. The elastic band is removed from your arm.

Once the blood has been collected, the needle is removed, and the puncture site is covered to stop any bleeding. In infants or young children, a sharp tool called a lancet may be used to puncture the skin and make it bleed. The blood collects into a small glass tube called a pipette, or onto a slide or test strip. A bandage may be placed over the area if there is any bleeding.

The blood is then analyzed in a laboratory to determine how certain antibodies react with specific antigens. The test can be used to confirm the identity of the specific microorganism. There are several serology techniques that can be used depending on the suspected antibodies. Serology techniques include agglutination, precipitation, complement-fixation, fluorescent antibodies, and others.

### **How the Test Will Feel**

When the needle is inserted to draw blood, some people feel moderate pain, while others feel only a prick or stinging sensation. Afterward, there may be some throbbing.

### **Why the Test is Performed**

A serology test can determine if you have ever been exposed to a particular microorganism, but this does not necessarily indicate a current infection.

### **Normal Results**

Normally, no antibodies are found in the blood sample.

### **What Abnormal Results Mean**

Detection of antibodies can be used to either diagnose an active or previous infection, or to determine if you are immune to reinfection by an organism. As the disease gets worse, more antibodies will be present. If a disease is suspected, the test may need to be repeated 10 days to 2 weeks after the first test.

If antibodies are found, you may:

- Have a current infection
- Have been infected in the past
- Have immunity to a certain organism and are unlikely to become sick

Some of the different diseases that can be detected include:

- Amebiasis
- Anthrax
- Brucellosis
- Human immunodeficiency virus (HIV)

- Fungal infection
- Measles
- Rubella
- Viral hepatitis (various types)

### Blood serum

In blood, the serum is the component that is neither a blood cell nor a clotting factor; it is the blood plasma with the fibrinogens removed. Serum includes all proteins not used in blood clotting and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms). The study of serum is serology. Serum is used in numerous diagnostic tests, as well as in blood typing. This formula can be applied: Serum = plasma - fibrinogens (and other clotting proteins)

### Blood plasma

**Blood plasma** is the yellow liquid component of blood, in which the blood cells in whole blood would normally be suspended. It makes up about 55% of the total blood volume. It is mostly water (90% by volume) and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide (plasma being the main medium for excretory product transportation). Blood plasma is prepared by spinning a tube of fresh blood in a centrifuge until the blood cells fall to the bottom of the tube. The blood plasma is then poured or drawn off. Blood plasma has a density of approximately  $1025 \text{ kg/m}^3$ , or  $1.025 \text{ kg/l}$ .

### Vaccine

A **vaccine** is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe. The agent 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 any of these microorganisms that it later encounters. Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g. vaccines against cancer are also being investigated; see cancer vaccine).

## Types

There are several types of vaccines currently in use. These represent different strategies used to try to reduce risk of illness, while retaining the ability to induce a beneficial immune response.

### A-Killed

Vaccines containing killed microorganisms – these are previously virulent micro-organisms which have been killed with chemicals or heat. Examples are the [Influenza \(flu\)](#), [cholera](#), [bubonic plague](#), [polio](#) and [hepatitis A](#) vaccines.

### B- Attenuated

Some vaccines contain live, [attenuated](#) microorganisms. Many of these are live [viri](#) that have been cultivated under conditions that disable their virulent properties, or which use closely-related but less dangerous organisms to produce a broad immune response, however some are bacterial in nature. They typically provoke more durable immunological responses and are the preferred type for healthy adults. Examples include the viral diseases [yellow fever](#), [measles](#), [rubella](#), and [mumps](#) and the bacterial disease [typhoid](#). The live Mycobacterium [tuberculosis](#) vaccine developed by Calmette and Guérin is not made of a [contagious](#) strain, but contains a virulently modified strain called "[BCG](#)" used to elicit immunogenicity to the vaccine.

### C- Toxoid

[Toxoids](#) – these are inactivated toxic compounds in cases where these (rather than the micro-organism itself) cause illness. Examples of toxoid-based vaccines include [tetanus](#) and [diphtheria](#). Not all toxoids are for micro-organisms; for example, *Crotalus atrox* toxoid is used to vaccinate dogs against rattlesnake bites.

### D- Subunit

[Protein subunit](#) – rather than introducing an inactivated or attenuated micro-organism to an immune system (which would constitute a "whole-agent" vaccine), a fragment of it can create an immune response. Examples include the subunit vaccine against [Hepatitis B virus](#) that is composed of only the surface proteins of the virus (previously extracted from the [blood serum](#) of chronically infected patients, but now produced by [recombination](#) of the viral genes into [yeast](#)), the [virus-like particle](#) (VLP) vaccine

against [human papillomavirus](#) (HPV) that is composed of the viral major [capsid](#) protein, and the [hemagglutinin](#) and [neuraminidase](#) subunits of the [influenza](#) virus.

### E-Conjugate

[Conjugate](#) – certain bacteria have [polysaccharide](#) outer coats that are poorly [immunogenic](#). By linking these outer coats to proteins (e.g. toxins), the [immune system](#) can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the *Haemophilus influenzae* type B vaccine.

### F- Experimental

A number of innovative vaccines are also in development and in use:

- Dendritic cell vaccines combine [dendritic cells](#) with antigens in order to present the antigens to the body's white blood cells, thus stimulating an immune reaction. These vaccines have shown some positive preliminary results for treating brain tumors.
- [Recombinant](#) Vector – by combining the physiology of one micro-organism and the [DNA](#) of the other, immunity can be created against diseases that have complex infection processes
- [DNA vaccination](#) – in recent years a new type of vaccine called *DNA vaccination*, created from an infectious agent's DNA, has been developed. It works by insertion (and [expression](#), triggering immune system recognition) of viral or bacterial DNA into human or animal cells. Some cells of the immune system that recognize the proteins expressed will mount an attack against these proteins and cells expressing them. Because these cells live for a very long time, if the [pathogen](#) that normally expresses these proteins is encountered at a later time, they will be attacked instantly by the immune system. One advantage of DNA vaccines is that they are very easy to produce and store. As of 2006, DNA vaccination is still experimental.
- [T-cell receptor](#) peptide vaccines are under development for several diseases using models of [Valley Fever](#), [stomatitis](#), and [atopic dermatitis](#). These peptides have been shown to modulate [cytokine](#) production and improve cell mediated immunity.
- Targeting of identified bacterial proteins that are involved in complement inhibition would neutralize the key bacterial virulence mechanism.

While most vaccines are created using inactivated or attenuated compounds from micro-organisms, [synthetic vaccines](#) are composed mainly or wholly of synthetic peptides, carbohydrates or antigens.

## G-Valence

Vaccines may be monovalent (also called univalent) or multivalent (also called polyvalent). A monovalent vaccine is designed to immunize against a single antigen or single microorganism. A multivalent or polyvalent vaccine is designed to immunize against two or more strains of the same microorganism, or against two or more microorganisms. In certain cases a monovalent vaccine may be preferable for rapidly developing a strong immune response.

## SEROLOGIC DIAGNOSTIC METHODS

### Introduction

Infectious diseases can be definitively diagnosed in only three ways:

1. By documenting the presence in the patient of an agent known to cause the disease, either by visualizing the agent directly in clinical material obtained from the patient, by detecting antigens or genetic material specific for the agent, or by cultivating the agent in the lab.
2. By detecting a specific product of the infectious agent in clinical material obtained from the patient, a product that could not be produced without the agent's presence.
3. By detecting an immunological response specific to the infecting agent in the patient's serum.

### Antigen - antibody reactions.

Antigen - antibody reactions are the methods by which antigens and antibodies are measured.

Antibodies can be detected by various methods from the serum. Many types of immunoglobulin molecules can be produced in response to a single antigen and detected either total Ig or Ig classes, as IgM, IgG and IgA.

Antigen - antibody reaction can be visualized in different ways according to the type of the antigen, conditions of the reaction and the medium the reaction takes place in.

When an antibody combines with a corpuscular antigen (forming part of a cell - e.g. bacteria, virus, blood cell or inert part with bound antigen) the cells **agglutinate**, that means they form clumps.

When an antibody combines with a noncorpuscular antigen (toxin, enzyme, microbial extract) a **precipitate** is formed, antigen - antibody complex is thrown out of solution.

When an antibody combines with an antigen which forms part of the surface of certain cells (e.g. a few species of gram negative bacilli and red blood cells) and provided complement is also present the cells are **lysed**, that means they are dissolved.

Other types of serological reactions used for antigen or antibody detection are used in microbiology - e.g. **immunofluorescent test**, **EIA**, **ELISA** (enzyme immunosorbent assays), **RIA** (radioimmunoassay), which use specific detection systems. Serological tests are used for indirect diagnosing the infectious diseases and for prevention and prophylaxis of them as well.

#### Serological tests can be used in two ways:

1. A known antibody can be used to detect and measure an unknown antigen
2. A known antigen can be used to detect and measure an unknown antibody.

Antibody production is a dynamic process changing during the disease. Therefore examination of the single serum sample is not sufficient. To diagnose acute infectious disease the first blood sample must be taken in the beginning of the disease, the second one after 10 or 14 days. Both blood samples are examined at the same time under the same laboratory conditions. The first serum sample is kept in the deep-freeze.

Quantitative results are normally expressed in terms of **the titre of the serum**, the highest dilution of the serum at which a particular effect can be demonstrated. For example a titer of 1:128 means that in the reaction being studied the serum shows the effect when is diluted 1 in 128. The titre is thus a measure of the amount of antibody in a unit volume of the original serum.

Acute infectious disease is diagnosed by appearance of four-fold increase of antibody titer in the second serum sample (e.g. titer 1:8 in the first serum sample and the titer 1:32 in the second one).

#### Components in the serological reactions.

##### A/ Serum

Is taken after the blood coagulation into the sterile tube. The full blood can coagulate itself or can be centrifuged 10 minutes at 800g. Serum can be exhausted after blood coagulation by Pasteur pipette or by plastic tip on the automatic pipette. Serum samples can be examined either native or are taken in a special tubes in the deep-freeze at -20oC. In some reactions the serum must be inactivated 30 min at +56oC before examination. Inactivation removes the serum complement.

##### B/ Antigen

**1. Corpuscular antigens** are mostly suspensions of inactivated bacteria taken from the solid culture medium.

**2. Noncorpuscular antigens** are the filtrates of bacterial cultures taken from the fluid culture medium. They can be also taken from the bacteria destroyed by sonication or by repeating freezing and thawing.

##### C/ Vehiculum

Serologic reactions are carried out in a special medium, either in solution or agar and under an optimum temperature.

**D/ Incubation time**

Varies from a few minutes (serotypization of bacteria) to several days (agar precipitation).

**Serologic tests are performed as:**

**A/ qualitative tests** - find out presence of antibodies or antigens in the serum

**B/ quantitative tests** - are performed more often. Serum is diluted in a physiological buffer in twofold or tenfold dilutions. Quantitative results are normally expressed in terms of the titre of the serum.

Formerly the serologic tests were performed in the tubes. Now most of the tests are performed as micromodifications. Instead of the tubes the plastic microtubes are used for storage, inactivation or predilution of the sera, then the sera are tested in the wells of a microplate. 25  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l or 200  $\mu$ l of a serum is used. The other laboratory equipment being used for microtests are automatic micropipettes with plastic tips, automatic dilutors, automatic washers and readers. In several laboratories the readers are in line with computers and at the end of the reaction it is possible to get the final result printed on the laboratory slip.

**Types of serologic reactions.****A. Agglutination**

**Agglutination** is the clumping of particles. The word *agglutination* comes from the Latin *agglutinare*, meaning "to glue to."

This occurs in biology in three main examples:

1. The clumping of cells such as bacteria or red blood cells in the presence of an antibody. The antibody or other molecule binds multiple particles and joins them, creating a large complex.
2. The coalescing of small particles that are suspended in solution; these larger masses are then (usually) precipitated.
3. An allergic reaction type occurrence where cells become more compacted together to prevent foreign materials entering them. This is usually the result of an antigen in the vicinity of the cells.

A corpuscular antigen - **agglutinogen** - is agglutinated when the specific antibody - **agglutinin** - is added. Agglutination can be read either visually or in the microscope . Presence or absence of clumping is noted.

**A/Direct agglutination**

Tests the presence of antibodies in the serum. It is used most often as **Widal reaction** for diagnosing



typhus and paratyphus. Direct agglutination is also used for detecting antibodies in tularemia, brucellosis (**Wright reaction**), listeriosis, rickettsial disease (**Weil-Felix reaction**).

### **B/ Indirect agglutination**

Tests the presence of unknown microbial antigen structure with a diagnostic serum. **Slide agglutination** is most often used method for the identification of the bacteria by means of specific diagnostic sera, especially in enterobacteria. Specific antisera are prepared by immunization of animals with bacterial strain.

### **C/ Co agglutination**

Specific immunoglobulin G (e.g. against *Streptococcus* spp., *Haemophilus influenzae*, *Neisseria meningitidis*) is bound with Fc fragment to protein A which is present on the surface of bacterial cell wall of *Staphylococcus aureus*, strain Cowan I. After addition of the antigen the clumps are formed on the slide within 1 minute.

This reaction is used for identifying serological types of *Streptococcus* spp., *Neisseria meningitidis* and *Haemophilus influenzae*.

### **D/ Direct haemagglutination**

Is an agglutination of red blood cells. Haemagglutination can be caused by antierythrocytic antibodies, by several viruses (e.g. myxoviruses, paramyxoviruses) and bacteria (*Bordetella pertussis*) which contain antigen called **haemagglutinin**. Haemagglutination is caused after their binding on the receptors present on the erythrocytal surface.

Direct haemagglutination is used for the diagnosing of infectious mononucleosis (**Paul-Bunnell reaction**), for the detection of cold agglutinins in atypical pneumonia caused by *Mycoplasma pneumoniae* (agglutination of human group O red blood cells at low temperatures).

### **E/ Haemagglutination - inhibition test**

Is used in virology. Many viruses agglutinate red blood cells because of containing haemagglutinin . Since the process is specifically inhibited by antibodies against the virus, haemagglutination inhibition can be used as a test for identifying viruses and measuring antibodies.

**F/ Indirect (passive) haemagglutination**

Red blood cells which are first treated with tannic acid or formalin are the carriers of soluble antigens. Antigen is either adsorbed or bound on the erythrocyte surface.

The method is sensitive and is used to detect antibodies against enterobacteria, *Clostridium tetani*, *Treponema pallidum* (TPHA test) and against some tissue antigens (e.g. thyroid gland antigens).

**G/ Latex agglutination**

Inert latex particles are the carriers of antigens. There are many commercial latex particle tests, e.g. for grouping streptococci, detecting bacterial antigens from cerebrospinal fluid, for detecting rheumatoid factors, for detecting some viruses from obtained samples (rotaviruses and adenoviruses from the stools). Also antibodies against rubella can be detected by latex particle test.

**B. Precipitation**

When a specific antibody - **precipitin** combines with a colloidal antigen - **precipitinogen** in solution or in gel the antigen - antibody complex is thrown out of solution - **precipitate**. The precipitate is most heavy in the **equivalence zone**, when antigen and antibody are fully combined. In some tests optimum relation between antigen and antibody must be kept up to carry out the reaction - the so called **flocculation**. Flocculation test is used for the quantitative measurement of toxin, toxoid or antitoxin. Precipitation reactions may be carried out in various ways:

**A/ Ring precipitation test**

A solution of antigen is layered on the surface of the antibody in a small tube or capillary tube. A narrow ring of precipitate occurs near the junction of two fluids. The result can be read visually. The concentration of immunoprecipitate is possible to be measured by this method or by laser nephelometry.

This type of test is used for grouping streptococci (according to C polysaccharide), for determining unknown proteins in forensic medicine.

**B/ Slide precipitation**

Is carried out on a slide and the occurrence of precipitate is detected in the light microscope. This type of precipitation test is used for diagnosing of lues (**quick reagin reaction**).

**C/ Gel - diffusion precipitation**

Antigen and antibody meet in an agar medium and a thin line of precipitate is produced there (antigen - antibody complex).

**1. Single diffusion**

Antigen diffuses in the agar medium (antibody is homogenously spread in the agar). It is carried out either in the tubes - **single gel - diffusion by Oudin** or on the slide - **single radial immunodiffusion by Mancini**.

The principle of the reaction: The antigen is placed in a well cut in an agar gel containing suitable diluted antibody. A ring of precipitate forms where the reactants meet in optimal proportions. The higher is the concentration of the examined antigen, the greater is the diameter of the ring. According to the diameter of the ring it is possible to count the concentration of the examined antigen.

This type of immunodiffusion is used for quantitative determination of immunoglobulins (IgM, IgG, IgA and IgD), complement components and other serum proteins.

## **2. Double immunodiffusion by Ouchterlony**

Is used more often. Antigen and antibody are allowed to diffuse towards each other in an agar medium, e.g. from separate wells cut in an agar plate or in a Petri dish. When antigen and antibody meet in optimal proportions they produce a thin line of precipitate. Position of the precipitate line depends on concentrations of both antigen and antibody and on their diffusion coefficient.

This reaction is used for diagnosing various bacterial, viral, fungal and autoimmune diseases, for recognizing toxin production by *Corynebacterium diphtheriae*.

## **D/ Immunoelectrophoresis**

Is a combination of electrophoresis and gel - diffusion precipitation. Antigens (most usually serum proteins) are first divided by electrophoresis according to their electric charge (albumins are directed towards the anode and globulins towards the cathode) on an agar coated slide. After electrophoresis is finished the longitudinal troughs are cut in the agar parallel to the axis of electrophoresis and filled with antibody. Diffusion then takes place. When antigen and antibody meet precipitate lines of single immunoglobulin classes occur. The lines are read after staining by amidoblack dye. Immunoelectrophoresis is a delicate technique for analyzing complicated mixtures of antigens and antibodies, e.g. serum immunoglobulins.

## **E/ Countercurrent immunoelectrophoresis**

Is a rapid and more sensitive variant of double diffusion method in which an electric current is used to drive the antigen towards the antibody in negatively charged gel.

This method was used to detect hepatitis B surface antigen. It is used for the rapid detection of bacterial antigens in clinical specimens, alfa-1-fetoprotein, etc. This method is being replaced by the ELISA methods.

## Complement fixation test

The **complement fixation test** is an **immunological medical** test that can be used to detect the presence of either specific **antibody** or specific **antigen** in a patient's **serum**. It was widely used to diagnose infections, particularly with microbes that are not easily detected by culture methods, and in rheumatic diseases. However, in clinical diagnostics labs it has been largely superseded by other serological methods such as **ELISA** and by DNA-based methods of pathogen detection, particularly **PCR**.

### Process

The CF test uses sheep **red blood cells** (sRBC), pre-bound by anti-sRBC antibody, and serum (usually from guinea pig) as a source of **complement**, which is a system of serum proteins that react with antigen-antibody complexes. If this reaction occurs on a cell surface, it will result in the formation of trans-membrane pores and therefore destruction of the cell. Accordingly, if the antibody-sensitized sRBC are brought into contact with active complement, they will undergo disintegration (hemolysis).

Complement will also react with antigen-antibody complexes in solution. The complement is thereby expended and can no longer trigger hemolysis; inhibition of complement hemolysis therefore indicates the presence of antigen-antibody complexes. A patient's serum containing a certain antibody (specific for, say, rubella virus) will yield antigen-antibody complexes after addition of the corresponding antigen (inactivated rubella virus in our example). Complement added to the mixture will be consumed, and sensitized sRBC added subsequently will not undergo hemolysis. Therefore, absence of hemolysis constitutes a positive CF test (patient's serum contains the antibody of interest).

### Immunofluorescence

**Immunofluorescence** is the labeling of **antibodies** or **antigens** with **fluorescent dyes**. This technique is often used to visualize the subcellular distribution of **biomolecules** of interest. Immunofluorescent-labeled tissue sections or cultures are studied using a **fluorescence microscope** or by **confocal microscopy**.

### Uses

Most commonly, immunofluorescence employs two sets of antibodies: a primary antibody is used against the antigen of interest; a subsequent, secondary ("indirect"), dye-coupled antibody is introduced that recognizes the primary antibody. As with most fluorescence techniques, a significant problem with immunofluorescence is **photobleaching**. Loss of activity caused by photobleaching can be controlled by reducing the intensity or time-span of light exposure, by increasing the concentration of fluorophores, or by employing more robust fluorophores that are less prone to bleaching (e.g. **Alexa Fluors** or **DyLight Fluors**).

Many uses of immunofluorescence have been outmoded by the development of **recombinant proteins** containing fluorescent protein domains, e.g. **green fluorescent protein** (GFP). Use of such "tagged" proteins allows much better localization and less disruption of protein function.

### **Direct fluorescent antibody**

**Direct fluorescent antibody** (DFA or dFA) (also known as "Direct immunofluorescence") is a laboratory test that uses **antibodies tagged** with **fluorescent dye** that can be used to detect the presence of **microorganisms**. This method offers straight-forward detection of antigens using fluorescently labeled antigen-specific antibodies. Because detection of the antigen in a substrate of patient sample (cellular smear, fluid or patient- inoculated culture medium) is the goal, DFA is seldom quantitative. This is the main test used to **detect rabies** in animals and requires the examination of brain tissue.

### **Indirect Fluorescent Antibody**

A form of fluorescent antibody technique commonly used to detect serum antibodies and immune complexes in tissues and microorganisms in specimens from patients with infectious diseases. The technique involves formation of an antigen-antibody complex which is labeled with fluorescein-conjugated anti-immunoglobulin antibody.

### **ELISA**

**Enzyme-linked immunosorbent assay**, also called **ELISA**, **enzyme immunoassay** or **EIA**, is a **biochemical** technique used mainly in **immunology** to detect the presence of an **antibody** or an **antigen** in a sample. The ELISA has been used as a **diagnostic** tool in medicine and **plant pathology**, as well as a **quality control** check in various industries. In simple terms, in ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme

can convert to some detectable signal. ELISAs traditionally utilize **chromogenic** substrates, though newer assays employ **fluorogenic** and **electrochemiluminescent** substrates enabling much higher sensitivity. Technically, some fluorescent and electrochemiluminescent assays of this type are not strictly ELISAs as they are not "enzyme linked" but instead linked to some non-enzyme light emitting reporter. As the general principles in these assays are the same, they are often grouped in the same category as ELISAs.

## Types

### 1."Indirect" ELISA

The steps of "indirect" ELISA for determining antibody concentrations are:

1. Apply a sample of known antigen of known concentration to a surface, often the well of a **microtiter plate**. The antigen is fixed to the surface to render it immobile. Simple adsorption of the protein to the plastic surface is usually sufficient. These samples of known antigen concentrations will constitute a **standard curve** used to calculate antigen concentrations of unknown samples. Note that the antigen itself may be an antibody.
2. A concentrated solution of non-interacting protein, such as **bovine serum albumin** (BSA) or **casein**, is added to all plate wells. This step is known as blocking, because the **serum** proteins block non-specific adsorption of other proteins to the plate.
3. The plate wells or other surface are then coated with **serum** samples of unknown antigen concentration, diluted into the same buffer used for the antigen standards. Since antigen immobilization in this step is due to non-specific adsorption, it is important for the total protein concentration to be similar to that of the antigen standards.
4. The plate is washed, and a detection antibody specific to the antigen of interest is applied to all plate wells. This antibody will only bind to immobilized antigen on the well surface, not to other serum proteins or the blocking proteins.
5. Secondary antibodies, which will bind to any remaining detection antibodies, are added to the wells. These secondary antibodies are conjugated to the substrate-specific enzyme. This step may be skipped if the detection antibody is conjugated to an enzyme.
6. Wash the plate, so that excess unbound enzyme-antibody conjugates are removed.
7. Apply a substrate which is converted by the enzyme to elicit a chromogenic or fluorogenic or electrochemical signal.

- View/quantify the result using a spectrophotometer, spectrofluorometer, or other optical/electrochemical device.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. A major disadvantage of the indirect ELISA is that the method of antigen immobilization is non-specific; any proteins in the sample will stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich ELISA provides a solution to this problem.

## 2.Sandwich ELISA

**A sandwich ELISA.** (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

## 3.Competitive ELISA

A third use of ELISA is through competitive binding. The steps for this ELISA are somewhat different than the first two examples:

- Unlabeled antibody is incubated in the presence of its antigen.
- These bound antibody/antigen complexes are then added to an antigen coated well.
- The plate is washed, so that unbound antibody is removed. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence "competition.")
- The secondary antibody, specific to the primary antibody is added. This second antibody is coupled to the enzyme.
- A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

For competitive ELISA, the higher the original antigen concentration, the weaker the eventual signal. The major advantage of a competitive ELISA is the ability to use crude or impure samples and still selectively bind any antigen that may be present.(Note that some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with your sample antigen (unlabeled). The more antigen in the sample, the less labeled antigen is retained in the well and the weaker the signal).






#### 4.Reverse ELISA

A new technique uses a solid phase made up of an immunosorbent polystyrene rod with 4-12 protruding ogives. The entire device is immersed in a test tube containing the collected sample and the following steps (washing, incubation in conjugate and incubation in chromogenous) are carried out by dipping the ogives in microwells of standard microplates pre-filled with reagents.

The advantages of this technique are as follows:

1. The ogives can each be sensitized to a different reagent, allowing the simultaneous detection of different antibodies and different antigens for multi-target assays;
2. The sample volume can be increased to improve the test sensitivity in clinical (saliva, urine), food (bulk milk, pooled eggs) and environmental (water) samples;
3. One ogive is left unsensitized to measure the non-specific reactions of the sample;
4. The use of laboratory supplies for dispensing sample aliquots, washing solution and reagents in microwells is not required, facilitating ready-to-use lab-kits and on-site kits.



- Reagents:
- Heat inactivated antibody-containing serum 
  - Heat inactivated unknown sample 
  - Guinea pig complement 
  - Purified antigen (Test system) 
  - Antibody-sensitized sheep erythrocytes (Indicator system) 

**Specificity**

- Controls:
- Red cells alone = no lysis
  - Antigen + Red cells = no lysis
  - Antibody + Red cells = no lysis
  - Unknown Sample + Red cells = no lysis
  - Complement + Red cells = lysis

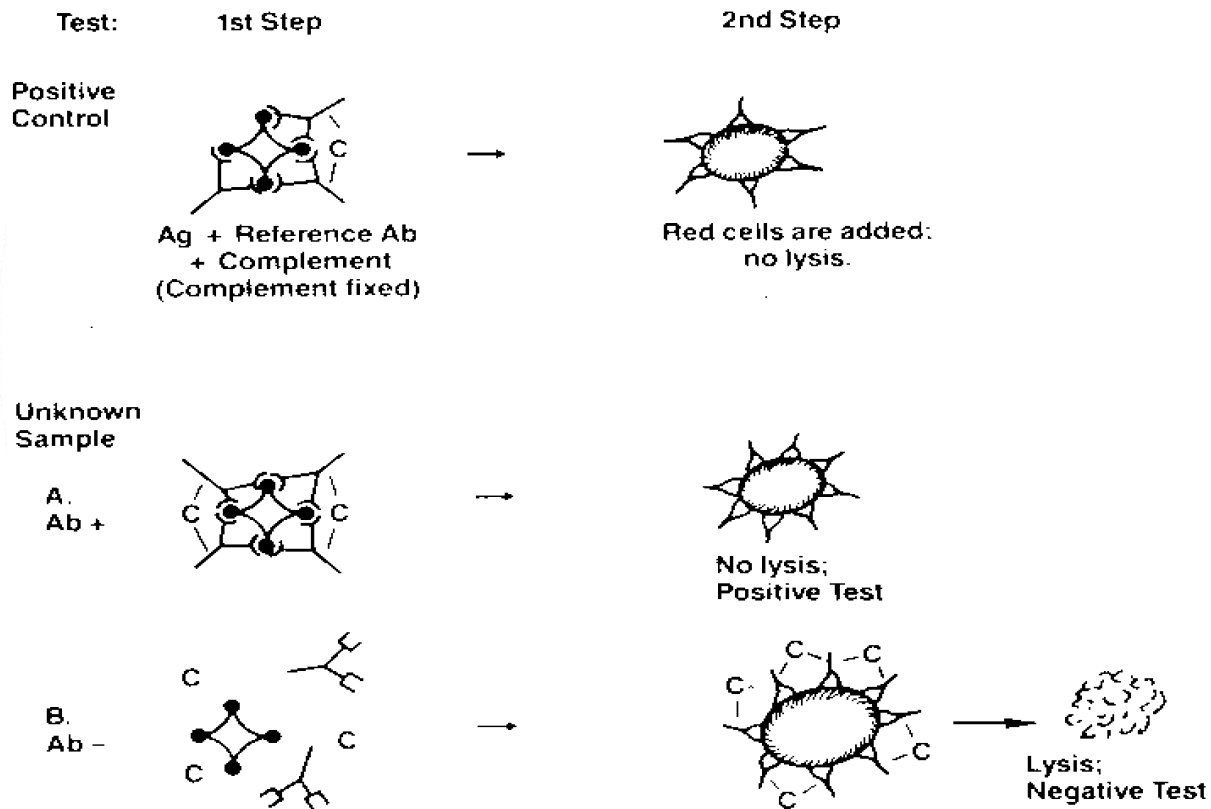


Figure 1: Diagrammatic representation of the general principles of complement fixation test

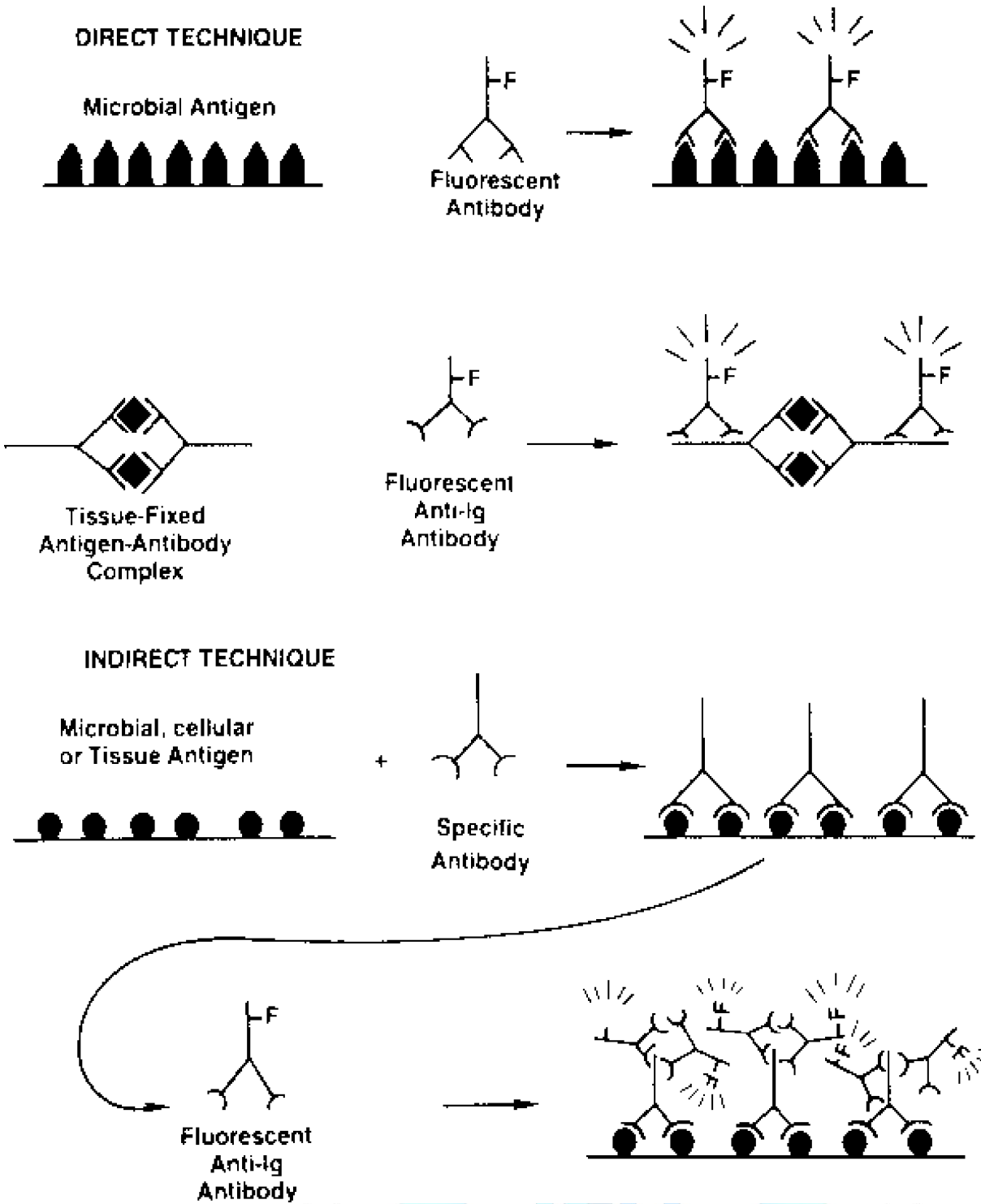


Figure 2 : Diagrammatic representation of the general principles of direct and indirect Immunofluorescence.

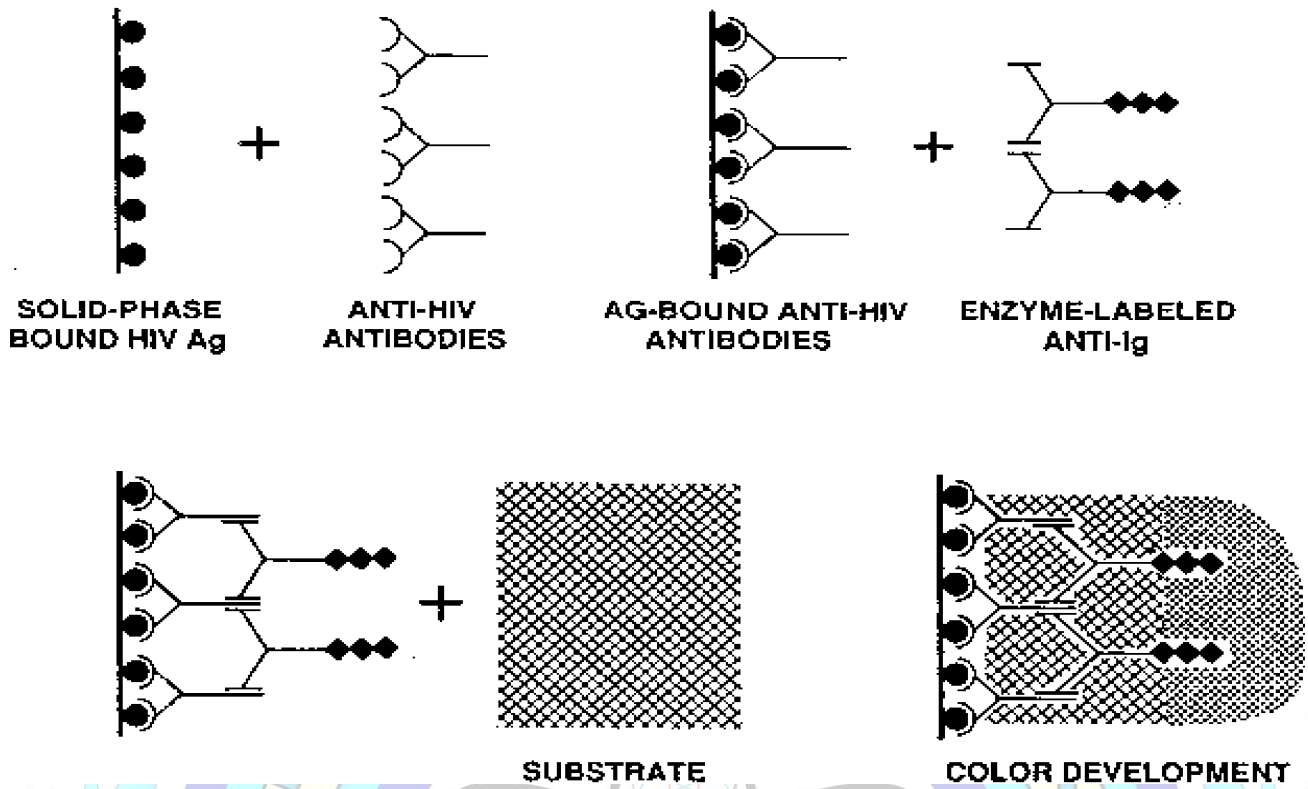


Figure 3 : Diagrammatic representation of an enzyme immunoassay test for the diagnosis of HIV infection

## Radioimmunoassay

A highly sensitive and specific assay method that uses the competition between radiolabeled and unlabeled substances in an antigen-antibody reaction to determine the concentration of the unlabeled substance; it can be used to determine antibody concentrations or to determine the concentration of any substance against which specific antibody can be produced.

**Radioimmunoassay** (RIA) is a very sensitive technique used to measure concentrations of **antigens** (for example, **hormone** levels in the **blood**) without the need to use a **bioassay**.

Although the RIA technique is extremely **sensitive** and extremely **specific**, it requires specialized equipment and is costly. It also requires special precautions, since radioactive substances are used. Therefore, today it has been largely supplanted by the **ELISA** method, where the antigen-antibody reaction is measured using **colorimetric** signals instead of a radioactive signal. The **RAST test** (radioallergosorbent test) is an example of radioimmunoassay. It is used to detect the causative **allergen** for an **allergy**.

### Method

To perform a radioimmunoassay, a known quantity of an **antigen** is made **radioactive**, frequently by labeling it with gamma-radioactive **isotopes** of **iodine** attached to **tyrosine**. This radiolabeled antigen is then mixed with a known amount of **antibody** for that antigen, and as a result, the two chemically bind to one another. Then, a sample of **serum** from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites.

As the **concentration** of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the **supernatant** is measured. Using known standards, a **binding curve** can then be generated which allows the amount of antigen in the patient's serum to be derived.

### RAST test

A **RAST test** (short for **radioallergosorbent test**) is a blood test used to determine to what substances a person is **allergic**. This is different from a **skin allergy test**, which determines allergy by the reaction of a person's skin to different substances.

### Indication

The RAST test is an alternative to skin tests to elucidate the causal allergen to an allergy.

Advantages of the RAST test range from: improved sensitivity without loss of specificity, to excellent reproducibility across the full measuring range of the calibration curve. In general, this method of blood testing (in-vitro, out of body) vs skin-prick testing (in-vivo, in body) has a major advantage: it is not always necessary to remove the patient from an antihistamine medication regimen, and if the skin conditions (such as **eczema**) are so widespread that allergy skin testing can not be done. Still, when possible, allergy skin testing is the preferred method in comparison with various *in vitro* tests for assessing the presence of specific IgE antibodies because it is more sensitive and specific, simpler to use, and less expensive.

### Method

The RAST test is a **radioimmunoassay test** to detect specific **IgE** antibodies to suspected or known **allergens**. IgE is the **antibody** associated with **Type I allergic response**: for example, if a person exhibits a high level of IgE directed against **pollen**, the test may indicate the person is allergic to pollen (or pollen-like) proteins. A person who has outgrown an allergy may still have positive IgE years after exposure.

The suspected allergen is bound to an insoluble material and the patient's serum is added. If the serum contains antibodies to the allergen, those antibodies will bind to the allergen. Radiolabeled anti-human IgE antibody is added where it binds to those IgE antibodies already bound to the insoluble material. The unbound anti-human IgE antibodies are washed away. The amount of radioactivity is proportional to the serum IgE for the allergen.

### Nephelometry and turbidimetry

Nephelometry is a technique used in immunology to determine levels of IgM, IgG, and IgA. It is performed by measuring the reduction in the intensity of the incident light after it passes through the sample being measured. In nephelometry the measurement is made by measuring the light passed through a sample at an angle.

This technique is widely used in clinical laboratories because it is relatively easily automated. It is based on the principle that a dilute suspension of small particles will scatter light (usually a laser) passed through it rather than simply absorbing it. The amount of scatter is determined by collecting the light at an angle (usually about 70 or 75 degrees).

Antibody and the antigen are mixed in concentrations such that only small aggregates are formed that do not quickly settle to the bottom. The amount of light scatter is measured and compared to the amount of scatter from known mixtures. The amount of the unknown is determined from a standard curve.

The nephelometric quantification is based upon the specific reaction of a monospecific anti-IgG subclass-specific antiserum with the human IgG subclass to be determined. The generated immune complexes are quantified by measuring the side-scattered light. A distinct advantage of nephelometry is the relatively short incubation time. As another advantage, nephelometry assays may be readily automated and are therefore suitable for the routine measurement of IgG subclasses in large numbers of samples.

Fixed time-nephelometry is illustrated in figure 9.

Brief outline of the method:

- Diluted samples (serum, plasma or other biological fluids), standard-and control sera are introduced in the reaction tubes of the nephelometer;
- Appropriate anti-IgG subclass reagents and reaction buffer are added;
- Side-scattered light is recorded;
- IgG subclass concentrations in the test samples are calculated relative to the calibration curves, obtained with the nephelometric IgG subclass standard serum;
- A control serum is assayed to check the validity of the calibration curves and the accuracy of the IgG subclass determinations.

Turbidimetry is similar to nephelometry in that it is based upon the fluid-phase optical detection of antigen complexes. However, in turbidimetry the decrease in light transmission is recorded, rather than the side-scattered light, which is measured in nephelometry.

### **Radial Immunodiffusion (RID)**

RID (Mancini) is a classical diagnostic method to determine IgG subclasses. This reliable assay is widely used and easy to perform. However, when large numbers of samples have to be processed the relatively long incubation times will be a disadvantage.

#### **Brief outline of the method:**

The RID assay is performed in (ready-for-use) agar plates, containing the specific anti-IgG subclass antibodies. Test samples, standard-and control sera are prepared and added to the plates. After 48-64 hours incubation at room temperature the diameters of the immunoprecipitation rings are measured. The IgG subclass concentrations in the test samples may be quantified in two ways:

- a) Calibration curve method: ring diameters and concentrations of the standards are plotted and the values of the test sample are determined by interpolation.
- b) Tabular method: ring diameters of the calibration curve are listed and the values of the test sample are read from a table. It is not necessary to make a calibration curve. The control serum is assayed to check the validity of the calibration curves and also the accuracy of the IgG subclass quantification, when using the table.

#### **Immuno-affinity chromatography**

In immuno-affinity chromatography the IgG subclasses are determined by monoclonal anti-IgG subclass-specific antibodies, bound to a solid phase in an open column. The captured IgG subclass molecules are labelled with a polyclonal, fluorescently-labelled anti-IgG reagent. The amount of bound conjugate is proportional to the amount of captured IgG subclass. The conjugate is eluted from the column and subsequently measured in a fluorescence reader. The IgG subclass concentrations of the test samples are calculated using the calibration curves.

Comparison of the most frequently used assays

Comparison of the RID-, nephelometry and ELISA assays for determination of human IgG subclass levels

Assay	Detection Range	Inter assay variation	Automation	Assay time	Work load
RID	ug/ml	small	no	long (>48 hours)	moderate
Nephelometry	ug/ml	very small	complete	very short (few minutes)	minimal
ELISA	ng/ml	medium	partly	Short (few hours)	high

Immunoelectrophoresis

**Immunoelectrophoresis** is a general name for a number of biochemical methods for separation and characterization of **proteins** based on **electrophoresis** and reaction with **antibodies**. All variants of immunoelectrophoresis require **immunoglobulins**, also known as **antibodies** reacting with the proteins to be separated or characterized. The methods were developed and used extensively during the second half of the 20th century. In somewhat chronological order: Immunoelectrophoretic analysis (one-dimensional immunoelectrophoresis *ad modum* Grabar), crossed immunoelectrophoresis (two-dimensional quantitative immunoelectrophoresis *ad modum* Clarke and Freeman or *ad modum* Laurell), rocket-immunoelectrophoresis (one-dimensional quantitative immunoelectrophoresis *ad modum* Laurell), fused rocket immunoelectrophoresis *ad modum* Svendsen and Harboe, **affinity immunoelectrophoresis** *ad modum* Bøg-Hansen.

Immunization

**Immunization**, or **immunisation**, is the process by which an individual's **immune system** becomes fortified against an agent (known as the **immunogen**). When an immune system is exposed to **molecules** that are foreign to the body (*non-self*), it will orchestrate an immune response, but it can also develop the ability to quickly respond to a subsequent encounter (through **immunological memory**). This is a function of the **adaptive immune system**. Therefore, by exposing an animal to an immunogen in a controlled way, its body can learn to protect itself: this is called active immunization.



The most important elements of the immune system that are improved by immunization are the **B cells** (and the **antibodies** they produce) and **T cells**. **Memory B cell** and **memory T cells** are responsible for a swift response to a second encounter with a foreign molecule. Passive immunization is when these elements are introduced directly into the body, instead of when the body itself has to make these elements. Immunization can be done through various techniques, most commonly **vaccination**. Vaccines against **microorganisms** that cause **diseases** can prepare the body's immune system, thus helping to fight or prevent an **infection**. The fact that **mutations** can cause **cancer cells** to produce proteins or other molecules that are unknown to the body forms the theoretical basis for therapeutic **cancer vaccines**. Other molecules can be used for immunization as well, for example in experimental vaccines against **nicotine** (NicVAX) or the hormone **ghrelin** (in experiments to create an obesity vaccine).

### **Passive and active immunization**

Immunization can be achieved in an active or passive fashion: vaccination is an active form of immunization.

### **Active immunization**

Active immunization entails the introduction of a foreign molecule into the body, which causes the body itself to generate immunity against the target. This immunity comes from the T cells and the B cells with their antibodies. Active immunization can occur naturally when a person comes in contact with, for example, a microbe. If the person has not yet come into contact with the **microbe** and has no pre-made antibodies for defense (like in passive immunization), the person becomes immunized. The immune system will eventually create antibodies and other defenses against the microbe. The next time, the immune response against this microbe can be very efficient; this is the case in many of the childhood infections that a person only contracts once, but then is immune.

Artificial active immunization is where the microbe, or parts of it, is injected into the person before they are able to take it in naturally. If whole microbes are used, they are pre-treated, **attenuated vaccine**. Depending on the type of disease, this technique also works with dead microbes, parts of the microbe, or treated **toxins** from the microbe.

### **Passive immunization**

Passive immunization is where pre-synthesized elements of the immune system are transferred to a person so that the body does not need to produce these elements itself. Currently, **antibodies** can be used

for passive immunization. This method of immunization begins to work very quickly, but it is short lasting, because the antibodies are naturally broken down, and if there are no B cells to produce more antibodies, they will disappear. Passive immunization occurs physiologically, when **antibodies** are transferred from mother to **fetus** during **pregnancy**, to protect the fetus before, as well as shortly after birth.

Artificial passive immunization is normally administered by **injection** and is used if there has been a recent outbreak of a particular disease or as an emergency treatment for toxicity (for example, for **tetanus**). The antibodies can be produced in animals ("serum therapy") although there is a high chance of **anaphylactic shock** because of immunity against animal serum itself. Thus, **humanized antibodies** produced *in vitro* by **cell culture** are used instead if available.

**List of vaccine ingredients**

Vaccine	Culture media	Excipients
<b>Anthrax vaccine</b>	Puziss-Wright medium 1095, synthetic or semisynthetic	Aluminum Hydroxide, Amino Acids, Benzethonium Chloride, Formaldehyde or Formalin, Inorganic Salts and Sugars, Vitamins
<b>BCG (Bacillus Calmette-Guérin)</b>	Synthetic or semisynthetic	Asparagine, Citric Acid, Lactose, Glycerin, Iron Ammonium Citrate, Magnesium Sulfate, Potassium Phosphate
<b>DT (diphtheria vaccine plus tetanus vaccine)</b>	Synthetic or semisynthetic	Aluminum Potassium Sulfate, Bovine Extract, Formaldehyde or Formalin, Thimerosal (multi-dose) or Thimerosal* (single-dose)
<b>Hep A Hepatitis A vaccine</b>	Human diploid tissue culture, MRC-5	Aluminum Hydroxide, Amino Acids, Formaldehyde or Formalin, MRC-5 Cellular Protein, Neomycin Sulfate, 2-Phenoxyethanol, Phosphate Buffers, Polysorbate

Hep B Hepatitis B vaccine	Yeast or yeast extract	Aluminum Hydroxide, Phosphate Buffers, Thimerosal*, Yeast Protein
Human Papillomavirus (HPV)	Yeast or yeast extract	Amino Acids, Amorphous Aluminum Hydroxyphosphate Sulfate, Carbohydrates, L-histidine, Mineral Salts, Polysorbate 80, Sodium Borate, Vitamins
Influenza vaccine	Chicken embryo	Beta-Propiolactone, Calcium Chloride, Neomycin, Ovalbumin, Polymyxin B, Potassium Chloride, Potassium Phosphate, Sodium Phosphate, Sodium Taurodeoxychoalate.
IPV Polio vaccine	Monkey kidney tissue culture (Vero cell)	Calf Serum Protein, Formaldehyde or Formalin, Monkey Kidney Tissue, Neomycin, 2-Phenoxyethanol, Polymyxin B, Streptomycin,
Japanese encephalitis vaccine (JE-Vax)	Mouse brain culture	Formaldehyde or Formalin, Gelatin, Mouse Serum Protein, Polysorbate 80, Thimerosal
Meningococcal vaccine	Mueller-Miller medium, Mueller-Minton medium, Watson-Scherp medium	Formaldehyde or Formalin, Phosphate Buffers Meningococcal (Menomune) Lactose, Thimerosal (10-dose vials only)
Pneumococcal vaccine	Bovine protein	Bovine Protein, Phenol
Rabies vaccine	Human dilpoid tissue culture, MRC-5	Human Serum Albumin, Beta-Propiolactone, MRC-5 Cellular Protein, Neomycin, Phenol Red (Phenolsulfonphthalein), Vitamins
Rotavirus vaccine	Monkey kidney tissue culture (Vero)	Cell Culture Media, Fetal Bovine Serum, Sodium Citrate, Sodium Phosphate

		Monobasic Monohydrate, Sodium Hydroxide Sucrose, Polysorbate 80
Typhoid (inactivated –Typhim Vi), Typhus vaccine	Synthetic or semisynthetic	Disodium Phosphate, Monosodium Phosphate, Phenol, Polydimethylsiloxane, Hexadecyltrimethylammonium Bromide
Typhoid (oral – Ty21a), Typhus vaccine	Bovine protein	Amino Acids, Ascorbic Acid, Bovine Protein, Casein, Dextrose, Galactose, Gelatin, Lactose, Magnesium Stearate, Sucrose, Yeast Extract
Varicella vaccine	Human diploid tissue cultures, MRC-5 and WI-38	Bovine Albumin or Serum, Ethylenediamine-Tetraacetic Acid Sodium (EDTA), Gelatin, Monosodium L-Glutamate, MRC-5 DNA and Cellular Protein, Neomycin, Potassium Chloride, Potassium Phosphate Monobasic, Sodium Phosphate Monobasic, Sucrose
Yellow fever vaccine	Chicken embryo	Egg Protein, Gelatin, Sorbitol
Zoster vaccine	Human diploid tissue cultures, MRC-5, and WI-38	Bovine Calf Serum, Hydrolyzed Porcine Gelatin, Monosodium L-glutamate, MRC-5 DNA and Cellular Protein, Neomycin, Potassium Phosphate Monobasic, Potassium Chloride, Sodium Phosphate Dibasic, Sucrose

## 1-Vaccine Strategies

The major strategies in developing vaccine are:

### 1-1 Whole Organism

The use of whole organism vaccination is the oldest strategy of vaccine development .Jenner s smallpox vaccine used live cowpox virus (vaccinia) to generate protective immunity .There are two classes of a whole organism vaccine ( Attenuated and Inactivated killed ).

### 1.2 Attenuated ( Live ) Vaccine

An attenuated whole organism vaccine uses a non pathogenic form of the desired microorganism. Non-pathogenicity may be induced by growing the pathogen in abnormal conditions .The advantage of the attenuated vaccine is that the attenuated pathogen stimulate an infection without conferring the disease .Since the microorganism is still living ,it provides continue antigenic stimulation giving sufficient time memory cell production .Example for attenuated vaccine .BCG –Bacillus Calmette Guerin for tuberculosis ,the Sabin vaccine for polio ,the measles vaccine ,the mumps and rubella vaccine.

### 1.3 Inactivated (Killed)

An activated whole organism vaccine uses pathogens which are killed and are no longer capable of replication within the host .The pathogens are inactivated by heat or chemical means while assuring that the surface antigens are intact .Inactivated vaccines are generally safe ,but are not entirely risk free example ( pertussis,influenza and Salk )vaccine.

### 1.4 Purified (Subunit ) Antigen

Avoided if only those parts of the pathogen which are necessary to elicit the proper immune response are used .In this way ,potential toxins may be avoided , or materials which inappropriately obscure or dominate an immune response may be removed .The antigenic properties of the various potential subunits of the pathogen must be examined in detail to determine which particular combinations will produce an effective immune response within the correct way .Often a response can be elicited , but there is no guarantee that immunogenic memory will be formed in corrected manner (Haemophilus influenza type B).

### 1.5 Recombinant Antigen

With the development of recombinant DNA techniques in the past few decades, other organism can be recruited and altered to produce the desired antigens more quickly, efficiently and safely .The first commercial vaccine of this type, Hepatitis B .Originally the primary components of hepatitis

vaccines, HBV –soluble antigen, had to be purified from a limited number of chronic human carriers of the virus. This was a costly method, and required extensive testing of the extract to prevent the presence of active, infectious viral particles .Using recombinant techniques, non pathogenic bacteria or yeasts were altered to produce the HBV-soluble antigen.

### **1.6 Synthetic Peptide**

The synthetic peptide approach to vaccine development in response to rapid DNA cloning and sequencing technology .Synthetic peptide vaccines are particularly useful in inducing the generation of memory helper T cells . This vaccine has had an impact on three major front, First, Bacteria synthetic peptide vaccine , for bacteria disease have focused primarily on trying to neutralize toxins ( diphtheria and cholera toxin ) . Second, Viruses –currently invariant regions are the focus of synthetic peptide vaccine design for viruses ( HIV protein ).Third ,Parasites scientist hope to achieve stage specific anti-malaria immunity by construction synthetic peptide vaccine for key malaria sporozoite epitopes.

### **1.7 Recombinant Vector**

T-cell only recognize antigens processed and presented in the context of the MHC molecule. Endogenous antigen is produced with in the host cell after bacterial or viral infection and is presented on MHC class 1 . This processing pathway is dependent upon the infection of the host cell by the pathogen .In order to overcome the inability of inactivated whole, purified antigen and recombinant antigen vaccines to enter cells, recombinant vector vaccines are used. Both humoral and cell mediated immune responses can be induced by the vector. Current research investigates the use of vaccinia for immunization against such pathogens such as hepatitis B virus ,herpes simplex and influenza virus .

### **1.8 DNA Vaccine**

Injection of plasmid DNA directly into recipient cells has been show to elicit a strong immune response .This method induces a prolonged expression of the DNA encoded peptide which has been shown to promote antibodies ,CD8+ and CD4+ T-cell that differentiate towards the Th1and Th2 response.

Different studies about the administration of "naked" plasmid DNA encoding a specific protein antigen was reported that could be induce expression of the protein in mouse, elicit antibodies against the protein and protect via cytolysis T cell response against the expressed protein . DNA vaccination provides the following advantage over protein vaccines, its greater chemical stability , relatively easy of purification and characterization , inherent adjuvant effect by unmethylated CPG dinucleotide motifs ,

direct entry of antigen in to intracellular MHC class I processing pathway facilitating CTL induction , intracellular antigen synthesis with posttranslational modification producing native tertiary antigen structure . Similarly , DNA vaccination provides advantages compared with recombinant viral vaccines as follow , relatively easy of construction , production & quality control , less risk of insertion mutagenesis , absence of vector so specific immune response that limit the efficacy of booster immunization , absence of risk related to recombinant events leading to converted of pathogenic viruses. So, Nucleic acid vaccine, similar to live attenuated vaccines , usually elicit both antibody and cytotoxic T – lymphocyte response .

## 2-Viral Vaccine (ex :- HBV)

### 2.1 Passive Immunization:-

Passively acquired anti HBs antibody could protect individuals from acute and chronic HBV infection if given soon after exposure to virus led to the development of a specific immunoglobulin containing high titers of anti – HBs. This HBIG was used before HB vaccine became available and recommended often in combination with HB vaccine, as post exposure prophylaxis following prenatal exposure for an infant born to HBs Ag positive mother, percutaneous or mucous membrane exposure to HBs Ag – positive blood, or by sexual contact with person who is positive for HBs Ag . HBIG is prepared from serum containing high titers of anti – HBs and is standardized to 100,000 IU of Anti – HBs per ml , HBIG is approximately 75% effective in preventing clinical hepatitis or the development of the carriers state if used shortly after exposure .

### 2.2 Hepatitis B vaccine:-

The availability of high safe and effective of hepatitis B vaccines now makes possible the establishment of programs aimed to eventual elimination or hepatitis B as a disease in man and the prevention of the first human cancer by a program of immunization. World wide strategies for hepatitis B prevention will differ from area to area according to the epidemiology of HBV infection.

Hepatitis B vaccine was introduced in the early 1980, and contains the small envelope proteins of the virus, which assembles as particles 22nm in diameter. Additional envelope proteins (Pre S1 and Pre S2) are then presented in vaccine. The S protein contain the principal envelope antigen , hepatitis B surface Ag (HBsAg) which is made up a common group specific determinat ("a") and several allelic subtype determinates. Antibodies to HBs Ag (anti HBs) are neutralizing , because of the shred ("a") determinate epitopes and cross protective among different subtypes . However, single nucleotide substitutions in the S genes may alter expression of the "a" determinate, possibly resulting in a reduction of neutralization by anti-HBs.

Newborn infants are now routinely vaccinated for hepatitis B. The series of immunization consists of three injections of the hepatitis B antigen given over a period of 6 months. When injected into the recipient, the antigen causes the host to make antibodies against it. The host, then having antibodies against hepatitis B surface antigen, when exposed with the entire Hepatitis B particle the host is become immune against infection from Hepatitis B .

The children vaccinated at birth to prevent prenatal HBV infection have shown that a continue high level of protection from chronic HBV infections persists at least 5 years. Hepatitis B vaccine should be administered intramuscularly in three doses of 0.5 ml (10 µg) each dose. The first dose should be given concurrently with HBIG but at a different site. If vaccine is not available at birth, the first vaccine dose may be given within 7 days of birth. The second and third doses should be given 1 month and 6 months) interval. Now, the type of vaccine which used in Iraq is hepatitis B vaccine recombinant (Euvax B).

### **2.3 Type of HBV Vaccine:-**

Two general types of HBV vaccine have been used widely.

#### **1- Plasma Derived Vaccine:-**

Plasma derived vaccines was first developed and licensed in 1981, (Heptavax B). Although, this type of vaccine is still used extensively in many countries it has been replaced then by recombinant vaccine. The inability to culture the virus has forced vaccines researches return to the early work of Krugman and associated workers (1973) they showed that immunity against HBV infection could be induced by injection of plasma containing hepatitis B surface antigen (HBsAg) .

plasma derived vaccine was prepared by harvested the 22 nm particles of HBs Ag from plasma of infected person , the particles are highly purified and any residual infections particles were inactivated by various combination of pepsin ,formaldehyde and heat. The expressed HBs Ag polypeptide self assemble into immunogenic spherical closely resembling that natural 22-nm particle found in the serum of people with chronic HBV infection. The final product consists of purified 22 nm HBs Ag particles. Aluminum hydroxide (alum) is used as an adjuvant.

#### **2- Recombinant DNA Vaccines :**

Recombinant DNA vaccines are produced by inserting plasmids containing HBs Ag genes into yeast or mammalian cells. All licensed vaccines consist of the 266 amino acid S gene product (HBs Ag protein), except for GENHEVAC B, which consist of 281 amino acid Pre S2 + Pre S gene product. The yeast production vaccine, which are the most widely used, are obtained by inserting the gene of HBs Ag into the plasmid downstream of three genes and *Sacchoromyces cervices* that services to product the



antigen .The HBs Ag must then be purified to eliminate yeast component was done by various physical separation techniques including chromatography and filtration.

The expressed HBs Ag polypeptide self assembles into immunogenic spherical particles closely resembling to the natural 22-nm particles found in the serum of people with chronic HBV infection.

### **3- Combination Vaccine :-**

Several vaccine manufactures are working to produce combination vaccines containing Hepatitis B component ,Diphtheria ,Tetanus and Pertusis (DTP-HepB) ;hepatitis B; Haemophilus, influenzae b(Hib) ;and inactivated poliovirus are also under development ( DTaP-Hep B Hib ; and DTP –Hep B-Hib-IPV). In addition, new recombinant vaccine containing both pre S1 and S2 antigens produced in yeast or stably transformed mammalian cell line are being developed in the hope that they may be more immunogenic, particular in persons who do not response to convention vaccines. Some studies try to used the Salmonella –vector and a nucleic acid vaccine for hepatitis B may have the advantage of efficiently inducing virus specific cytotoxic T cell as well as neutralizing antibodies.

### **2.4 Hazard of Immunization:-**

Numerous studies indicate that HB vaccines has an excellent safety profile and that serious adverse events after HB vaccination are exceeding rare .Only mild injection – site reactions occur in 22 percent of immunized persons but fever and other systemic symptoms are uncommon . Anaphylaxis is rare but does occur. The Gullen – Bar Syndrome has been reported in vaccine recipients but the cause uncertain. In other studies showed that adverse events reported occurs after HB vaccination includes Gullain – Bar Syndrome and multiple sclerosis. In general, these events are rarely, occur in the absence of HB vaccination and have their peck incidence in the older age's groups . The only contraindication the administration of vaccine in person with hypersensitivity to yeast or to a component of the vaccine.

Hepatitis B vaccines are safe to administer to adult and children. More than an estimated 10 million adults and 2 million infants and children have been vaccinated in the United States, and at least 12 million children have - been vaccinated worldwide, only one case of anaphylaxis occurred among 100,763 children ages (10-11) years who had been vaccinated with recombinant vaccine in British Columbia. No adverse events were reported among 166,757 children who had been vaccinated with plasma-derived vaccine in New Zealand.

### **2.5 Immune Response to HB Vaccine:-**

More than a decade of experience in hepatitis B vaccination has shown that the immune response to the hepatitis B vaccine in healthy subjects is extremely variable. Following administration of 3 doses of vaccine under optimal conditions, most recipients produce a high-titered and long-lasting anti-HBs response. However, (5 to 10) % of healthy vaccine's respond slowly and poorly (anti-HBs titer  $\leq$  100

mIU/ml) and maintain protective antibody levels ( $\geq 10$  mIU/ml) for only very limited periods. Approximately (1 to 3) % of neonates and up to 10% of healthy adults do not produce adequate levels ( $<10$  mIU/ml) of antibodies after 3 doses of the currently available vaccines, and can be considered non responders.

### Newer strategies for vaccine development

The advent of molecular biology and genetic engineering, as in every other domain of biology, has had a dramatic effect on vaccine development, providing greater opportunities for construction of inactivated antigens and for rational attenuation of organisms through directed mutation. Table 1 lists some of the newer strategies that depend on molecular biology. The first success of genetic engineering was a hepatitis B vaccine manufactured in a yeast recombinant carrying the gene for the S protein, which replaced a vaccine based on purification of S particles from plasma of infected individuals. Subsequently, insertion of genes into yeast, *Escherichia coli* or Chinese hamster ovary cells enabled production of a variety of recombinant proteins, such as Lyme OspA, cytomegalovirus gB and pertussis toxin. Recombinants of viruses and bacteria also may be used as live vaccines, on condition that they are apathogenic. For example, bovine or attenuated human parainfluenza 3 viruses can serve as the backbone for insertion of genes from other parainfluenza viruses or from respiratory syncytial virus, and attenuated yellow fever virus can serve as the carrier for genes from dengue or West Nile viruses. Protein expression by the inserted genes is immunizing.

Table 1 Newer strategies for vaccine development starting from microbial DNA, cDNA or RNA

Strategy	Examples of pathogens targeted
Recombinant protein production	Hepatitis B S <sub>Ag</sub> , pertussis toxin, Lyme outer surface protein A, CMV gB protein
Live recombinants carrying genes from related agents	Dengue genes in yellow fever 17D, parainfluenza 1 + 2 genes in parainfluenza 3, <i>M. tuberculosis</i> genes in BCG
Recombinant vectors recombining genes from pathogens	HIV, CMV
Alpha virus replicons	HIV, Hemorrhagic Fevers
Replication-defective particles	HPV, SARS
'Naked' DNA plasmids	HIV and many others
Prime boost using DNA and/or	HIV, malaria, tuberculosis

vectors	
Reverse vaccinology	Meningococcus B
Microarrays for expression of virulence genes	Mainly bacteria
Synthetic peptides	Cancer, CTL vaccines
Synthetic capsular polysaccharides	Hib
Reverse genetics	Influenza, parainfluenza, RSV
Hib, <i>H. influenza</i> type b; IPV, inactivated polio vaccine; T, tetanus; d, adult diphtheria dose; CMV, cytomegalovirus; HPV, human papillomavirus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; HIV, human immunodeficiency virus; CTL, cytotoxic T lymphocyte.	

### **Immunology finally helps vaccinology**

It must be admitted that until recently immunology has not contributed much to the development of vaccines. Most successes in immunization have been mediated through the induction of protective antibodies, whereas the major challenges now facing us (e.g., HIV, malaria, tuberculosis) will require the induction of T cell immunity as well. Fortunately, several of the new strategies, including vectors, plasmid DNA and lipidated peptides, are capable of inducing both CD4+ and CD8+ cellular responses. In addition, the paucity of adjuvants for vaccines, until recently essentially limited to aluminum salts that stimulate a T helper type 2 (TH2) response, is at last being corrected by the creation of new oil-in-water emulsions, liposomes, Toll-like receptor agonists, cytokines and other substances that push the immune system in a T helper type 1 (TH1) direction. Moreover, immunologists have recently provided us with tests for cellular immunity that can be done on a large scale, such as ELISPOT assays for cytokine induction and tetramer staining for CD8+ cell peptide specificity. The recent rediscovery of T regulatory cells may also have an impact on vaccines for pathogens that try to evade the immune system.

### **New means and new ends**

In the early days of the twenty-first century, one can descry several notable tendencies in vaccine development. Combinations of vaccines have become ever more necessary as new components become part of routine vaccination. Already hexavalent combinations containing diphtheria, tetanus, pertussis, *H. influenzae* type b (Hib), hepatitis B and inactivated polio vaccines are used in Europe, and pentavalent combinations in many other parts of the world. Varicella vaccine has been incorporated into measles-mumps-rubella vaccines, and various combinations of *H. influenzae* type b, pneumococcal and

meningococcal conjugated bacterial polysaccharide vaccines will become available. Another easily discerned tendency is toward the stimulation of innate as well as adaptive immune responses. This can be accomplished by the choice of proper adjuvants such as CpG oligonucleotides, which stimulate both types of responses. Proteomics will probably advance to the point of allowing construction in vitro of proteins with more natural conformations, and polysaccharide synthesis is just beginning to be practical. Whereas vaccination is usually considered as prophylaxis, serious attempts are being made to develop therapeutic vaccines for chronic infections. The basic idea is to induce cellular immune responses that suppress infection, even when the host has been unable to mount those responses naturally. Examples include immunization against the E6 and E7 oncogenes of papillomaviruses for the treatment of cervical cancer, and against the gag and tat genes of HIV for the suppression of viral replication in AIDS. A very important part of the future is the enlargement of routes of immunization (Table 2). Most vaccines today are given by parenteral injection, which induces systemic immune responses expressed by B and T cells in the blood. But the need for mucosal immune responses has become increasingly obvious. The new live, attenuated influenza vaccine is given intranasally, induces both systemic and local responses and gives a broader protection against antigenically drifted strains. Aerosol administration of measles and rubella vaccines implants the attenuated viruses at the natural sites of replication and elicits immunity equivalent to that after injection. The aerosol route could lend itself to mass immunization using inhalation devices. Oral immunization has been used for some time to immunize with living organisms that replicate in the intestine, such as oral polio and typhoid Ty21a vaccines. Now attempts are being made to induce mucosal responses with nonliving antigens. One approach is to develop oral vaccines from plants made transgenic for vaccine antigens. Demonstration of adequate immune responses in human is awaited. Immunization by rectal or vaginal application of antigens is also under investigation.

Table 2 Nonparenteral routes of administration

Route	Example of use
Intranasal	Live influenza
Aerosol	Measles
	Rubella
Oral	Plants transgenic for Hepatitis BsAg
Transcutaneous (patches, microneedles, powder)	Hepatitis B, anthrax

### Extension to noninfectious diseases

Active immunization has heretofore been largely confined to infectious diseases, with some use of desensitization to treat allergies. Now consideration is being given to immunization against a wide variety of noninfectious diseases. Most effort is being directed against cancers, in which novel cellular antigens are often present. Vaccine incorporating proteins or peptides from cancer antigens are in advanced trials, with promising results measured by prolongation of life. It is also intriguing that individuals with inherited mutations that predispose to cancer might be immunized prophylactically before cancer develops. Tolerization to autoantigens is being attempted in many autoimmune diseases, such as multiple sclerosis and diabetes mellitus. Better antigens for inducing IgG rather than IgE antibodies against allergens are in development. Contraception can be maintained by immunization against hormones. Atherosclerosis and Alzheimer disease can perhaps be controlled by immunization against cholesterol fractions or amyloid, respectively. Lastly, drug addictions, including nicotine, methamphetamine and cocaine, may be controllable by inducing antibodies that rapidly remove the drugs from the body.

### New targets

New populations are being targeted for vaccination, as summarized in Table 3. Until now, most vaccination has been directed at infants and children; but it has become increasingly clear that adolescents and adults also need universal immunizations. Aside from new recommendations for booster immunization with diphtheria-tetanus-acellular pertussis vaccine, the possible incorporation of vaccines against meningococci, papillomaviruses, Herpes simplex and cytomegalovirus into routine vaccination will require an adolescent immunization date to prevent, respectively, sepsis, cervical cancer, genital herpes and congenital infection. Adults currently receive influenza and pneumococcal vaccines, but vaccination may also come into play against varicella virus to prevent reactivation in the form of zoster. Also, during the course of their lives, adults may need vaccination during pregnancy, hospitalization and travel. An experimental Group B streptococcal vaccine is available to prevent transmission of the bacteria from mothers to neonates and pregnant women could be immunized against a number of other pathogens (e.g., pneumococci, respiratory syncytial virus) in order to transmit protective antibodies that will protect their newborns for some months. Antibiotic-resistant nosocomial bacteria are an increasing problem and a staphylococcal capsular polysaccharide vaccine is in a later stage of development for patients susceptible to secondary infections.

Table 3 New target groups for vaccination

Groups	Vaccine targets
Infants (combination vaccines)	Diphtheria, tetanus, acellular pertussis, <i>Hameophilus influenzae</i> type b, hepatitis B, inactivated polio vaccine
Adolescents	Tetanus, adult diphtheria dose, acellular pertussis, CMV, HPV, HSV-2
Adults	Zoster, HSV-2
Hospital patients	Staphylococcal, Candida
Pregnant women	Group B Streptococcus, RSV
Civil defense workers	New vaccinia, anthrax, plague, Ebola, etc.
Individuals with noninfectious diseases	Cancer, Alzheimer disease, dental caries, autoimmune disorders, drug addiction
Individuals with chronic infections (therapeutic vaccines)	HIV, HPV
<p>CMV, cytomegalovirus; HPV, human papillomavirus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; HIV, human immunodeficiency virus.</p>	

### Problems in vaccine development

There are many problems inherent in developing a good protective anti-viral vaccine. Among these are:

- Different types of virus may cause similar diseases -- e.g. the common cold. As a result, a single vaccine will not be possible against such a disease
- Antigenic drift and shift -- This is especially true of RNA viruses and those with segmented genomes
- Large animal reservoirs. If these occur, re-infection after elimination from the human population may occur
- Integration of viral DNA. Vaccines will not work on latent virions unless they express antigens on cell surface. In addition, if the vaccine virus integrates into host cell chromosomes, it may cause problems (This is, for example, a problem with the possible use of anti-HIV vaccines based on attenuated virus strains)

- Transmission from cell to cell via syncytia - This is a problem for potential AIDS vaccines since the virus may spread from cell to cell without the virus entering the circulation.
- Recombination and mutation of the vaccine virus in an attenuated vaccine.

### Major sites of viral infection

In order to develop a successful vaccine, certain characteristics of the viral infection must be known.

One of these is the site at which the virus enters the body. Three major sites may be defined:

- Infection via mucosal surfaces of the respiratory tract and gastro-intestinal tract.
  - Virus families in this group are: rhinoviruses; myxoviruses; coronaviruses; parainfluenzaviruses; respiratory syncytial viruses; rotaviruses
- Infection via mucosal surfaces followed by spread systemically via the blood and/or neurones to target organs.

Virus families in this group are: picornaviruses; measles virus; mumps virus; herpes simplex virus; varicella virus; hepatitis A and B viruses

- Infection via needles or insect bites, followed by spread to target organs:

Virus families in this group are hepatitis B virus; alphaviruses; flaviviruses; bunyaviruses

IgA-mediated local immunity is very important in types 1 and 2. There is little point in having a good neutralizing humoral antibody in the circulation when the virus replicates, for example, in the upper respiratory tract. Clearly, here secreted antibodies are important.

Thus, we need to know:

- Viral antigen(s) that elicit neutralizing antibody
- Cell surface antigen(s) that elicit neutralizing antibody
- The site of replication of the virus

**Vaccine Administration**

Appropriate vaccine administration is critical to vaccine effectiveness. The recommended site, route and dosage for each vaccine are based on clinical trials, practical experience and theoretical considerations.

**Preparation****Patient Preparation**

Patients should be prepared for vaccination with consideration for their age and stage of development. Parents/guardians and patients should be encouraged to take an active role before, during and after the administration of vaccines.

- **Screening** - All patients should be screened for contraindications and precautions for each scheduled vaccine. Many state immunization programs and other organizations have developed and make available standardized screening tools.

**Vaccine Safety & Risk Communication**

Parents/guardians and patients are exposed through the media to information about vaccines, some of which is inaccurate or misleading. Healthcare providers should be prepared to discuss the benefits and risks of vaccines using Vaccine Information Statements (VIS) and other reliable resources. Establishing an open dialogue provides a safe, trust building environment in which individuals can freely evaluate information, discuss vaccine concerns and make informed decisions regarding immunization.

**A traumatic Care**

Vaccine safety issues and the need for multiple injections have increased the concerns and anxiety associated with immunizations. Healthcare providers need to display confidence and establish an environment that promotes a sense of security and trust for the patient and family, utilizing a variety of techniques to minimize the stress and discomfort associated with receiving injections. This is particularly important when administering vaccines to children.

**Positioning & Comforting Restraint**

The healthcare provider must accommodate for the patient's comfort, safety, age, activity level, and the site of administration when considering patient positioning and restraint. For a child, the parent/guardian should be encouraged to hold the child during administration. If the parent is uncomfortable, another person may assist or the patient may be positioned safely on an examination table.

**Pain Control** Pain is a subjective phenomenon influenced by multiple factors, including an individual's age, anxiety level, previous healthcare experiences, and culture. Consideration for these factors is important as the provider develops a planned approach to management of injection pain.



- **Topical Anesthetics or a vapocoolant** :- spray may be applied to decrease pain at the injection site. These products should be used only for the ages recommended and as directed by the product manufacturer.

- **Analgesic Agents**: - A non-aspirin containing pain reliever may be considered to decrease discomfort and fever following vaccination. These products should be used only in age-appropriate doses.

- **Diversiory Techniques**: - Age-appropriate non pharmacologic techniques may provide distraction from pain associated with injections. Diversion can be accomplished through a variety of techniques.

- **Dual Administrators**: - Some providers favor the technique of two individuals simultaneously administering vaccines at separate sites. The premise is that this procedure may decrease anxiety from anticipation of the next injection(s). The effectiveness of this procedure in decreasing pain or stress associated with vaccine injections has not been evaluated.

### Infection Control

Healthcare providers should follow Standard Precautions to minimize the risks of spreading disease during vaccine administration.

- **Handwashing** - The single, most effective disease prevention activity is good handwashing. Hands should be washed thoroughly with soap and water or cleansed with an alcohol-based waterless antiseptic between patients, before vaccine preparation or any time hands become soiled, e.g. diapering, cleaning excreta.
- **Gloving** - Gloves are not required to be worn when administering vaccines unless the person administering the vaccine is likely to come into contact with potentially infectious body fluids or has open lesions on the hands. It is important to remember that gloves cannot prevent needlestick injuries.
- **Needle stick Injuries** should be reported immediately to the site supervisor, with appropriate care and follow-up given as directed by state/local guidelines. Safety needles or needle-free injection devices should be used if available to reduce the risk of injury.
- **Equipment Disposal** - Used needles should not be detached from syringes, recapped or cut before disposal. All used syringe/needle devices should be placed in puncture proof containers to prevent accidental needle sticks and reuse. Empty or expired vaccine vials are considered medical waste and should be disposed of according to state regulations.

### Vaccine Preparation

Proper vaccine handling and preparation is critical in maintaining the integrity of the vaccine during transfer from the manufacturer's vial to the syringe and ultimately to the patient.

### Equipment Selection

**Syringe Selection** - A separate needle and syringe should be used for each injection. A parenteral vaccine may be delivered in either a 1-mL or 3-mL syringe as long as the prescribed dosage is delivered.

**Needle Selection** - Vaccine must reach the desired tissue site for optimal immune response. Therefore, needle selection should be based upon the prescribed route, size of the individual, volume and viscosity of the vaccine, and injection technique. Typically, vaccines are not highly viscous, and therefore a fine gauge needle (22-25 gauge) can be used.

**Needle-Free Injection** - A new generation of needle-free vaccine delivery devices has been developed in an effort to decrease the risks of needle stick injuries to healthcare workers and to prevent improper reuse of syringes and needles.

**Inspecting Vaccine** - Each vaccine vial should be carefully inspected for damage or contamination prior to use. The expiration date printed on the vial or box should be checked. Vaccine can be used through the last day of the month indicated by the expiration date unless otherwise stated on the package labeling. Expired vaccine should never be used.

**Reconstitution** - Some vaccines are prepared in a lyophilized form that requires reconstitution, which should be done according to manufacturer guidelines. Diluent solutions vary; use only the specific diluent supplied for the vaccine. Once reconstituted, the vaccine must be either administered within the time guidelines provided by the manufacturer or discarded. Changing the needle after reconstitution of the vaccine is not necessary unless the needle has become contaminated or bent. Continue with standard medication preparation guidelines.

**Labeling** - Once a vaccine is drawn into a syringe, the content should be indicated on the syringe. There are a variety of methods for identifying or labeling syringes (e.g. keep syringes with the appropriate vaccine vials, place the syringes in a labeled partitioned tray, or use color coded labels or Preprinted labels).

### **Subcutaneous injection (Sub-Q or SC)**

Injections are administered into the fatty tissue found below the dermis and above muscle tissue.

#### **Site**

Subcutaneous tissue can be found all over the body. The usual sites for vaccine administration are the thigh (for infants <12 months of age) and the upper outer triceps of the arm (for persons >12 months of age). If necessary, the upper outer triceps area can be used to administer subcutaneous injections to infants.

#### **Needle Gauge & Length**

5/8-inch, 23- to 25-gauge needle

#### **Technique**

- Follow standard medication administration guidelines for site assessment/selection and site preparation.
- To avoid reaching the muscle, pinch up the fatty tissue, insert the needle at a 45° angle and inject the vaccine into the tissue.
- Withdraw the needle and apply light pressure to the injection site for several seconds with a dry cotton ball or gauze.

### **Intramuscular injections (IM)**

injections are administered into muscle tissue below the dermis and subcutaneous tissue.

#### **Site**

Although there are several IM injection sites on the body, the recommended IM sites for vaccine administration are the vastus lateralis muscle (anterolateral thigh) and the deltoid muscle (upper arm). The site depends on the age of the individual and the degree of muscle development.

#### **Needle Gauge**

22- to 25-gauge needle.

#### **Needle Length**

For all intramuscular injections, the needle should be long enough to reach the muscle mass and prevent vaccine from seeping into subcutaneous tissue, but not so long as to involve underlying nerves, blood vessels, or bone. The vaccinator should be familiar with the anatomy of the area into which the vaccine will be injected.

Decision on needle size and site of injection must be made for each person on the basis of the size of the muscle, the thickness of adipose tissue at the injection site, the volume of the material to be administered, injection technique, and the depth below the muscle surface into which the material is to be injected .

#### **Technique**

- Follow standard medication administration guidelines for site assessment/selection and site preparation.
- To avoid injection into subcutaneous tissue, spread the skin of the selected vaccine administration site taut between the thumb and forefinger, isolating the muscle. Another technique, acceptable mostly for pediatric and geriatric patients, is to grasp the tissue and "bunch up" the muscle.
- Insert the needle fully into the muscle at a 90° angle and inject the vaccine into the tissue.
- Withdraw the needle and apply light pressure to the injection site for several seconds with a dry cotton ball or gauze.

**Aspiration**

Aspiration is the process of pulling back on the plunger of the syringe prior to injection to ensure that the medication is not injected into a blood vessel. Although this practice is advocated by some experts, the procedure is not required because no large blood vessels exist at the recommended injection sites.

**Multiple Vaccinations**

When administering multiple vaccines, **NEVER** mix vaccines in the same Syringe unless approved for mixing by the Food and Drug Administration (FDA). If more than one vaccine must be administered in the same limb, the injection sites should be separated by 1-2 inches so that any local reactions can be differentiated. Vaccine doses range from 0.2 mL to 1 mL.

The recommended maximum volume of medication for an IM site, varies among references and depends on the muscle mass of the individual. However, administering two IM vaccines into the same muscle would not exceed any suggested volume ranges for either the vastus lateralis or the deltoid muscle in any age group. The option to also administer a subcutaneous vaccine into the same limb, if necessary, is acceptable since a different tissue site is involved.

If a vaccine and an immune globulin preparation are administered simultaneously (e.g., Td/Tdap and tetanus immune globulin [TIG] or hepatitis B vaccine and hepatitis B immune globulin [HBIG]), a separate anatomic site should be used for each injection. The location of each injection should be documented in the patient's medical record.

**Special Situations****Bleeding Disorders**

Individuals with a bleeding disorder or who are receiving anticoagulant therapy may develop hematomas in IM injection sites. Prior to administration of IM vaccines the patient or family should be instructed about the risk of hematoma formation from the injection. Additionally, a physician familiar with the patient's bleeding disorder or therapy should be consulted regarding the safety of administration by this route. If the patient periodically receives hemophilia replacement factor or other similar therapy, IM vaccine administration should ideally be scheduled shortly after replacement therapy.

A 23-gauge or finer needle should be used and firm pressure applied to the site for at least 2 minutes. The site should not be rubbed or massaged.

**Latex Allergy** - Administration of a vaccine supplied in a vial or syringe that contains natural rubber (refer to product information) should not be administered to an individual with a history of a severe (anaphylactic) allergy to latex, unless the benefit of vaccination clearly outweighs the risk of an allergic

Reaction. These situations are rare. Medical consultation and direction should be sought regarding vaccination. A local or contact sensitivity to latex is not a contraindication to vaccination.

**Syncopal or Vasovagal Response** ("fainting") may occur during vaccine administration, especially with adolescents and adults. Because individuals may fall and sustain injury as a result, the provider should have the patient sit during injection(s). A syncopal or vasovagal response is not common and is not an allergic reaction. However, if syncope develops, the provider should observe and administer supportive care until the patient is recovered.

**Anaphylaxis** (a life-threatening acute allergic reaction) - Each facility that administers vaccines should have a protocol, procedures and equipment to provide initial care for suspected anaphylaxis. Facility staff should be prepared to recognize and respond appropriately to this type of emergency situation. All staff should maintain current CPR certification. Emergency protocols, procedures and equipment/supplies should be reviewed periodically. For additional information on medical management of vaccine reactions in children, teens, and adults. Although both fainting and allergic reactions are rare, vaccine providers should strongly consider observing patients for 15 minutes after they are vaccinated.

### **Documentation**

All vaccines administered should be fully documented in the patient's permanent medical record. Documentation should include: 1. Date of administration. 2. Name or common abbreviation of vaccine 3. Vaccine lot number 4. Vaccine manufacturer 5. Administration site 6. Vaccine Information Statement (VIS) edition date (found in the lower right corner of the back of the VIS). 7. Name and address of vaccine administrator. This should be the address where the record is kept. If immunizations are given in a shopping mall, for example, the address would be the clinic where The permanent record will reside.

## **GUIDELINE ON ADJUVANTS IN VACCINES**

### **1. INTRODUCTION**

Adjuvants (immune potentiators or immunomodulators) have been used for decades to improve the immune response to vaccine antigens. The incorporation of adjuvants into vaccine formulations is aimed at enhancing, accelerating and prolonging the specific immune response towards the desired response to vaccine antigens. Advantages of adjuvants include the enhancement of the immunogenicity of antigens, modification of the nature of the immune response, the reduction of the antigen amount needed for a successful immunization, the reduction of the frequency of booster immunizations needed and an improved immune response in elderly and immunocompromised vaccines.

Selectively, adjuvants can be employed to optimize a desired immune response, e.g. with respect to immunoglobulin classes and induction of cytotoxic or helper T lymphocyte responses. In addition, certain adjuvants can be used to promote antibody responses at mucosal surfaces.

Interest in vaccine adjuvants has been growing rapidly for several reasons.

Vaccine manufacturers and public health authorities, e.g. WHO, have established ambitious goals for enhancing present vaccines and for developing new ones, and new vaccine candidates have emerged over the past years against infectious, allergic and autoimmune diseases and also for cancer and fertility treatment.

In many cases, because of their low immunogenicity these vaccines require adjuvants. New technologies in the fields of analytical biochemistry, macromolecular purification, recombinant technology, and a better understanding of immunological mechanisms and disease pathogenesis have helped to improve the technical basis for adjuvant development and application.

Adjuvants can be classified according to their source (natural, synthetic or endogenous), mechanism of action, or physical or chemical properties. Adjuvant activity is a result of multiple factors and an enhanced immune response obtained with one antigen cannot as a rule be extrapolated to another antigen. Individual antigens vary in their physical, biological and immunogenic properties and antigens may have different needs for help from an adjuvant.

Adjuvants should be chosen based on the type of immune response desired and should be formulated with the antigen in such a way that the optimal type of response with the minimal side effects, is obtained. The major means by which adjuvants may exert their activities are: (i) presentation of the antigen, defined by the physical appearance of the antigen in the vaccine; (ii) antigen/adjuvant uptake; (iii) distribution (targeting to specific cells); (iv) immune potentiation/modulation which includes activities that regulate both quantitative and qualitative aspects of the ensuing immune responses; (v) the protection of the antigen from degradation and elimination.

In general, the mode of action of adsorbants and particulate adjuvants involves presentation of the antigen to the immune system, whereas the microbial, synthetic and endogenous adjuvants act by direct stimulation or modulation of the immune system. In addition to their role in the presentation of the antigen to the immune system, the mode of action of emulsions is to promote slow antigen release and protection from rapid elimination.

The use of repository adjuvants like mineral salts is accompanied by the formation of an inflammatory focus at the site of injection which may lead to the synthesis of pro-inflammatory cytokines and stimulation of innate immunity important for the initial steps of the immune response.

## **2. THE ADJUVANT**

### **2.1. Description**

The nature or chemical composition of the adjuvant should be described in detail. When more than one adjuvant is used and/or when an adjuvant has more than one component, the function of each adjuvant and/or each component should be described to the extent that it is known.

### **2.2. Manufacture**

The manufacture of the adjuvant should be described in detail. Special attention should be given to the source material for the adjuvant especially if this is biological in nature and any special considerations that may apply. Parameters that are critical in conferring the correct physical, biochemical, biological or adsorptive properties of the adjuvant should be defined. Attention should be paid to the use of any material of ruminant origin and if so, compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01) is required.

### **2.3. Characterization**

The results an assessment of a number of parameters used to characterise the adjuvant should be described. Critical parameters should be identified and described. Such parameters are likely to be part of the routine testing of batches of the adjuvant. Other parameters will also be analyzed to characterize the adjuvant and some of these may also form part of routine testing. The parameters which define an adjuvant will depend on the nature of the adjuvant and may include, but will not necessarily be limited to:

A-chemical composition (qualitative and quantitative)

B-physical characteristics (e.g., visual appearance, density, viscosity, pH, size and size distribution, surface charge)

C-biochemical characteristics

D-purity (e.g., endotoxin content, bioburden, manufacturing residuals)

### **2.4. Routine testing**

A list of tests to be applied routinely to the adjuvant should be defined as appropriate for the adjuvant in question and should be based on the parameters used to characterise the adjuvant as detailed above. Specifications should be set.

### **2.5. Stability**

Relevant physico-chemical and/or biological properties, based on the characteristics of the adjuvant, should be employed in assessing the stability of the adjuvant during storage. Stability indicating parameters may include structure and antigen adsorption/binding characteristics.

### **3. ADJUVANT/ANTIGEN COMBINATION**

#### **3.1. Development and manufacture of the combination**

Combining the antigen with the adjuvant is a crucial aspect of the final adjuvant-antigen combination. The mechanism of association and association efficiency between antigen and adjuvant should be defined and described. Aspects that are critical for the biological properties of the adjuvant-antigen combination (e.g. adsorption, binding characteristics) should be identified and monitored. If more than one adjuvant is to be incorporated, appropriate information for each adjuvant should be supplied and compatibility studies should be performed on the intended combination of adjuvant(s) and antigen(s).

The entire manufacturing process of the adjuvant-antigen combination should be described in detail.

An intermediate bulk may be formed during antigen and adjuvant combination, prior to formulation. In other cases, formulation will take place simultaneously with the adjuvant and antigen combination (final bulk). Alternatively combining the antigen with the adjuvant, formulation and filling (final product) may be a single process.

Any excipient or diluents added to the adjuvant-antigen combination during the preparation of the final bulk (formulation) should not adversely affect the potency of the vaccine or the association of the antigen(s) with the adjuvant(s).

In each case, the characterisation, routine testing and stability testing of the intermediate bulk, final bulk and the final product, where relevant, must be performed as detailed below. The vaccine manufacturer should clearly delineate and justify the tests that are being performed at each stage.

#### **3. 2. Characterization**

The adjuvant-antigen combination should be characterised as appropriate. This may include the level and consistency of association of the antigen with the adjuvant, the integrity of the antigen in association with the adjuvant, the effect of adjuvant on the ability to assay the antigen and the extent of release of the antigen from the adjuvant (stability). Other parameters may include chemical and physical characteristics (e.g., particle size, viscosity).

#### **3. 3. Routine testing**

Tests for routine verification of the adjuvant-antigen combination should be identified, described and validated. Such tests should be based on the parameters assessed during full characterization of the adjuvant-antigen combination.

#### **3.4. Stability**



The long-term stability of the adjuvant-antigen combination should be assessed investigating relevant physical and biochemical properties. The extent of dissociation of antigen from the adjuvant and its integrity may be important parameters.

### **3.5. Multiple antigen/adjuvant combinations**

If the final vaccine product comprises an antigen(s) in addition to the antigen present in the adjuvant-vaccine combination, then the effect of the adjuvant on this additional antigen(s) must be assessed using relevant tests for that antigen. Similarly, any effects of an additional antigen(s) on the adjuvant-antigen complex must be assessed. If the final vaccine product comprises more than one adjuvant-antigen combination, testing appropriate to the nature of the adjuvants (whether identical or not) will be required including any adverse effects occurring between the different adjuvant-antigen combinations.

## **4. FINAL PRODUCT**

The final vaccine product should be subjected to tests for potency, identity and stability.

## **DNA VACCINES**

### **The Third Vaccine Revolution**

These vaccines are based on the deliberate introduction of a DNA plasmid into the vaccinee. The plasmid carries a protein-coding gene that transfects cells *in vivo* at very low efficiency and expresses an antigen that causes an immune response. These are often called DNA vaccines but would better be called DNA-mediated or DNA-based immunization since it is not the purpose to raise antibodies against the DNA molecules themselves but to get the protein expressed by cells of the vaccinee. Usually, muscle cells do this since the plasmid is given intramuscularly. It should be noted that the plasmid does not replicate in the cells of the vaccinee, only protein is produced. It has also been shown that DNA can be introduced into tissues by bombarding the skin with DNA-coated gold particles. It is possible to introduce DNA into nasal tissue in nose drops. In the case of the gold bombardment method, one nanogram of DNA coated on gold produced an immune response. One microgram of DNA could potentially introduce a thousand different genes into the vaccinee.

### **Advantages of DNA vaccines**

- Plasmids are easily manufactured in large amounts
- DNA is very stable
- DNA resists temperature extremes and so storage and transport are straight forward

- A DNA sequence can be changed easily in the laboratory. This means that we can respond to changes in the infectious agent
- By using the plasmid in the vaccinee to code for antigen synthesis, the antigenic protein(s) that are produced are processed (post-translationally modified) in the same way as the proteins of the virus against which protection is to be produced. This makes a far better antigen than, for example, using a recombinant plasmid to produce an antigen in yeast (e.g. the HBV vaccine), purifying that protein and using it as an immunogen.
- Mixtures of plasmids could be used that encode many protein fragments from a virus or viruses so that a broad spectrum vaccine could be produced
- The plasmid 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. This also offers protection against diseases caused by certain obligate intracellular pathogens (e.g. Mycobacterium tuberculosis)

All of the above means that DNA vaccines are cheap and therefore likely to be developed against pathogens of lesser economic importance (at least to drug companies)

### Possible Problems

- Potential integration of plasmid into host genome leading to insertional mutagenesis
- Induction of autoimmune responses (e.g. pathogenic anti-DNA antibodies)
- Induction of immunologic tolerance (e.g. where the expression of the antigen in the host may lead to specific non-responsiveness to that antigen)

### Initial studies

Most work has been done on DNA vaccines against viruses since DNA-based plasmid immunization actually resembles virus infection. When they have been well-characterized, the immune responses are broad-based and mimic the situation seen in a normal infection by the homologous virus. The immune response can be remarkably long-lasting and even more so after one booster injection of plasmid. Cytotoxic T lymphocyte (CTL) responses are also well produced as might be expected since the immune system is seeing what is a model of an infected cell. One important demonstration using a DNA vaccine has been the induction of cytotoxic cellular immunity to a conserved internal protein of influenza A to determine if it might be possible to overcome the annual variation (antigenic drift and shift) of the virus.

CTLs were derived in mice against the conserved flu nucleoprotein and this was effective at protecting the mice against disease, even when they were challenged with a lethal dose of a virulent heterologous virus with a different surface hemagglutinin. Because transfer of anti-nucleoprotein antibodies to untreated mice does not protect them from disease, the protective effect of the vaccine must have been cell-mediated.

The current influenza vaccine is an inactivated preparation containing antigens from the flu strains that are predicted to infect during the next flu season. If such a prediction goes awry, the vaccine is of little use. It is the surface antigens that change as a result of reassortment of the virus in the animal (duck) reservoir (see [influenza](#)). The vaccine is injected intramuscularly and elicits an IgG response (humoral antibody in the circulation). The vaccine is protective because enough of the IgG gets across the mucosa of the lungs where it can bind and neutralize incoming virus by binding to surface antigens. If a plasmid-based DNA vaccine is used, both humoral and cytotoxic T lymphocytes are produced, which recognize antigens presented by plasmid-infected cells. The CTLs are produced because the infected muscle cells present flu antigens in association with MHC class I molecules. If the antigen presented is the nucleocapsid protein (which is a conserved protein), this overcomes the problem of antigenic variation. Such an approach could revolutionize the influenza vaccine. Other studies have used a mix of plasmids encoding both nucleoprotein and surface antigens. Protection by DNA vaccines has also been demonstrated with rabies, mycoplasma and *Plasmodium yoelii*. Anti-HIV vaccines are also being tested. In the [HIV](#) chapters, it was noted that progress on AIDS vaccines has been stymied by the fact that many vaccines only elicit humoral antibodies while the use of whole virus vaccines (which might elicit CTL responses) has been rejected because of other potential problems. Plasmid-based vaccines may overcome these problems.

### Systemic Immunization

Systemic immunization is the method of choice at present for most vaccinations. This is usually carried out by injecting the vaccine subcutaneously or intramuscularly into the deltoid muscle. Ideally all vaccines would be given soon after birth, but some are deliberately delayed, for various reasons. The common systemic vaccines for measles, mumps and rubella are usually given at 1 year of age because, if given earlier, maternal antibody would decrease their effectiveness. The carbohydrate vaccines for *Pneumococcus*, *Meningococcus* and *Haemophilus* infections are usually given at about 2 years of age as before this age they respond poorly to polysaccharides unless they are associated with protein components that can act to recruit T cell help for the development of anti-polysaccharide antibody, e.g. hen egg albumin .

### Mucosal immunization

Recent vaccination approaches have focused on the mucosal route as the site of choice for immunization either orally or through the nasal associated immune tissue . This is because most infectious agents gain entry to the systemic system through these routes and the largest source of lymphoid tissue is at the mucosal surfaces. Moreover, if successful it would obviate the need for, in some instances, painful injections and allow for the self-administration of certain vaccines such as those used for immunization against influenza. Adjuvant vaccines and live vectors have been used to target the mucosal immune system with some success. Attenuated strains of salmonella can act as a powerful immune stimulus as well as acting as carriers of foreign antigens. This approach has been used to immunize mucosal surfaces against herpes simplex virus and human papilloma virus. Furthermore, bacterial toxins, e.g. those derived from cholera, *E. coli* and *Bordetella pertussis*, have immunomodulatory properties and are thus being exploited in the development of mucosally active adjuvants. Pertussis toxin has been shown to augment the costimulatory molecules B-7 on B cells and CD28 on T cells as well as increasing IFN $\gamma$  production. Hopefully, oral and nasal vaccines may soon be available to obviate the need for the invasive techniques that are currently in use.

**Table 2 Passive immunization**

Infection	Source of antiserum	Indications
Tetanus	Immune human; horse	Post exposure (plus vaccine)
Diphtheria	Horse	Post-exposure
Gas gangrene	Horse	Post-exposure
Botulism	Horse	Post-exposure
Varicella-zoster	Immune human	Post-exposure in immunodeficiency
Rabies	Immune human	Post-exposure (plus vaccine)
Hepatitis B	Immune human	Post-exposure, prophylaxis
Hepatitis A	Pooled human Ig	Prophylaxis
Measles	Immune human	Post-exposure in infants
Snake bite	Horse	Post-bite
Some autoimmune diseases	Pooled human Ig	Acute thrombocytopenia and neutropenia

**Process for the preparation of a vaccine for the treatment of tuberculosis and other intracellular infections diseases and the vaccine produced by the process**

**1- A process for the preparation of a vaccine against an intracellular pathogen selected from the group consisting of M. tuberculosis, M. leprae, Leishmania, Salmonella, Trypanosoma, Plasmodium, Brucella, Listeria, and Streptococcus, wherein the process comprises the steps of:**

- (i) culturing the intracellular pathogen.
- (ii) culturing syngeneic (same strain), allogeneic (different strain) and xenogeneic (different species like sheep and goat) macrophages and macrophage cell lines selected from the group consisting of J774, P388D1, RAW, BMC-2 and THP-1;
- (iii) infecting the macrophages and macrophage cell lines with the selected pathogen of step (i);
- (iv) treating the infected macrophages and macrophage cell lines with pathogen specific drugs to kill the pathogen, followed by gamma irradiation to kill the macrophage or macrophage cell line and remaining pathogens to obtain a composition;
- (v) immunizing disease resistant and susceptible strains of animals with the composition;
- (vi) infecting the vaccinated animals with live selected pathogen and monitoring animal mortality, and viable counts of the pathogen in lungs, spleen and liver;
- (vii) monitoring the vaccinated animals for proliferation and generation of CD4\* Th1 and Th2 cells and CD8\* cytotoxic T cells indicating the generation of cell mediated immunity against the pathogen; and
  - (viii) wherein the composition is a vaccine if administration of the composition results in decreased mortality of vaccinated animals and/or decreased viable counts of the pathogen in lungs, spleen, and liver of the infected animals when compared to non-immunized animals.

**2. A process for the preparation of a vaccine against tuberculosis, the process comprising the steps of:**

- (i) culturing M. tuberculosis H37Rv;

- (ii) culturing syngeneic, allogeneic and xenogenic macrophages and macrophage cell lines selected from the group consisting of J774, P388D1, RAW, BMC-2 and THP-1;
- (iii) infecting the macrophages and macrophage cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *M. tuberculosis*;
- (iv) treating the infected macrophage and macrophage cell lines with amikacin, isoniazid and gamma irradiation to kill the macrophage or macrophage cell line and remaining *M. tuberculosis* to obtain a composition;
- (v) immunizing tuberculosis resistant and susceptible strains of mice with an allogeneic macrophage tuberculosis composition or syngeneic macrophage tuberculosis composition or xenogenic macrophages tuberculosis;
- (vi) infecting the vaccinated group of mice with live *M. tuberculosis* and monitoring animal mortality, and viable counts of *M. tuberculosis* in lungs, spleen and liver;
- (vii) monitoring the vaccinated animals for proliferation and generation of CD4\* Th1 and Th2 cells, and CD8\* cytotoxic T cells indicating the generation of cell mediated immunity against *M. tuberculosis*; and
- (viii) wherein the composition is a vaccine if administration of the composition results in decreased mortality of vaccinated animals and/or decreased viable counts of *M. tuberculosis* in lungs, spleen, and liver of the infected animals when compared to non-immunized animals.

**3. A process for the preparation of a vaccine against salmonella, the process comprising the steps of:**

- (i) culturing *Salmonella typhimurium*;
- (ii) culturing syngeneic, allogeneic macrophages and xenogenic macrophages and macrophage cell lines selected from the group consisting of J774, P388D1, RAW, BMC-2 and THP-1;

- (iii) infecting the macrophages and macrophage cell lines (J774, P388D1, RAW, RMC-2, THP-1) with *S. typhimurium*;
- (iv) treating the macrophage and macrophage cell lines with mitomycin C and gamma irradiation to kill the macrophage or macrophage cell line and remaining *S. typhimurium* to obtain a composition;
- (v) immunizing salmonella resistant and susceptible strains of mice with the composition;
- (vi) infecting the vaccinated group of mice with live *S. typhimurium* and monitoring animal mortality, and viable counts of *S. typhimurium* in lungs, spleen and liver,
- (vii) monitoring the vaccinated animals for proliferation and generation of CD4\* Th1 and Th2 cells, and CD8\* cytotoxic T cells indicating the generation of cell mediated immunity against *S. typhimurium*; and
- (viii) wherein the composition is a vaccine if administration of the composition results in decreased mortality of vaccinated animals and/or decreased viable counts of *S. typhimurium* in lungs, spleen, and liver of the infected animals when compared to non-immunized animals.