Unit III

General method of the preparation of bacterial vaccines, toxoids, viral vaccine, antitoxins, serum-immune blood derivatives and other products relative to immunity.

Storage conditions and stability of official vaccines

Vaccines

A vaccine is a pharmaceutical suspension or solution of biological origin (immunogenic substance) that provides active acquired immunity to a particular disease. Vaccines are antigen containing preparations and the process of active immunization by vaccines is known as vaccination. Vaccine is defined as biological and therapeutic agent because these vaccines derived particularly from pathogenic micro-organisms and are effective due to formation of antibodies by immunological reactions, these antibodies act on further infections of the particular pathogenic micro-organism.

The immunogenic material of vaccine is also known as antigenic material. Reaction of this immunological material with specific T-cell produces specific antibodies that are useful in prevention of further infection of particular micro-organism. The antigenic agent resembles a disease causing micro-organism and is made from weakened or killed form or toxic material or one of the surface protein of particular micro-organism.

Vaccines may be used as either prophylactic or therapeutic purpose. Worldwide eradication and restriction of various diseases quite possible because of the widespread immunity of vaccination like small pox, polio, measles and tetanus were eradicated from either whole or much of the world. The vaccination was first denoted by Edward Jenner for cow pox and vaccine was derived from variolae vaccinae (smallpox of cow). If a vaccinated individual develops a disease against which already vaccinated, it is likely to be less virulent than in unvaccinated persons. The general side effects associated with vaccines are fever, allergies, pain and muscle aches.

There are various types of vaccines depending upon their preparation, normally the toxin or microorganism is modified to destroy its toxicity and reduce it to a safe level without reducing its antigenicity.

1.Toxoids derived from bacterial exotoxin

Toxoids are derived from bacterial exotoxins. Toxoidal vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism. Examples of toxoid-based vaccines include tetanus and diphtheria. Toxoid vaccines are known for their efficacy. The bacterial toxins are incubated with formalin, this treatment completely destroy the toxic properties without altering their antigenic properties. The aim of toxoid preparation is to prepare a toxicity free, impurity free preparation while maintaining antigenicity.

Diphtheria toxoid

Diphtheria toxoid is prepared by formalin inactivation of diphtheria toxin. A suitable strain of Corynebacterium Diphtheriae is grown on a liquid medium which must not be made from the broth containing horse muscle otherwise the recipient will be sensitized to horse serum which may be dangerous to patient. When the toxin production is sufficient, the bulk organisms are removed and filtered and the filtrate is sterilized and formalin is added at 37 degree until the toxicity is removed upto two to three weeks. The resulting toxoid is known as Formol toxoid (Anatoxin) which was used for many years but often caused severe reactions is now replaced by widely used other forms of diphtheria vaccines. The reactions caused by crude formol toxoids have replaced them by toxoid –antitoxin Floccules (TAF). The 80 units of antitoxin are mixed with 100 units of Toxoid to neutralize the toxoid for three weeks the floccules are washed with saline and collected and resuspended in saline containing bactericide. The floccules are least likely to cause any reactions.

The purified formol toxoid is filtered and treated with alum which lead to precipitate containing aluminum hydroxide and phosphate, it forms a depot in tissues thus giving prolonged stimulus in body, therefore slow absorption and excretion increases the antigenic activity. This alum precipitated toxoid (APT) produces much higher levels of antibodies than formol toxoid and TAF, also it is free from sensitizing horse protein. Other more purified and freeze dried diphtheria toxoid is PTAP purified toxoid aluminium phosphate.

As per Ministry of health children should be vaccinated in first year for four diseases Diphtheria, pertussis, tetanus and poliomyelitis. But if each vaccine is given individually it would lead to a burdenand inconvenience to children therefore a combined vaccine for diphtheria, pertussis and teatanus (DPT vaccine) is designed.

DPT- Vaccine –DPT vaccine is a class of combination vaccines against three infectious diseases in humans: diphtheria, pertussis (whooping cough), and tetanus. The vaccine components include diphtheria and tetanus toxoids and killed whole cells of the bacterium that causes pertussis. Diphtheria and tetanus toxoids have been combined with pertussis antigens and used as a combination DPT vaccine since the 1940s. More recently, this DPT combination has been used as the basis for the development of combination vaccines containing additional vaccine antigens added singly or in additional combinations such as Haemophilus influenzae type b, hepatitis B and inactivated poliovirus, allowing multiple vaccine antigens to be delivered via a single injection. In addition, in some DTP vaccines the diphtheria dose has been reduced and pertussis antigen has been modified to allow these vaccines to be used for booster doses in adolescents and adults. Because these toxoids and antigens are now frequently used as combined vaccines, most adverse events following immunization reported in these vaccines are likely due to the safety profile of their individual components.

DPT Vaccine preparation: Multiple combination vaccines to prevent diphtheria, tetanus, and pertussis are in used globally and each has a specific composition.For diphtheria and tetanus the potency and amount of toxoid in a vaccine are recorded in International Units (IU) and in Limits of Flocculation. Whole-cell pertussis vaccines are standardised using a mouse protection test.

Usually it is available as a preparation adsorbed with aluminium hydroxide or phosphate and combined with other toxoids or vaccine antigens. The potency of diphtheria vaccine used for the immunization of children should not be less than 30 IU per single human dose, while for adults; the potency is about a third of the dose for children. Monovalent single antigen diphtheria toxoid is currently commercially unavailable.

Tetanus toxoid - Tetanus toxoid is a preparation of formalin inactivated toxin. This is prepared from exotoxin of clostridium tetani and this toxoid is available adsorbed with aluminium phosphate or hydroxide, alone or in combination with other toxoids or vaccines.

Staphylococcus Toxoid was made from exotoxin of staphylococcus aureus to prevent boils and staphylococcal infections; it has been replaced by antibiotic therapy now.

2. Suspensions of microorganisms

Suspension of microorganisms can be prepared from bacteria, rickettsia and viruses. They may be living or dead. These can be simple which are prepared by one species for example plague vaccine prepared by pasteurella pestis or mixed which are mixtures of two or more simple vaccines for example Typhoid and parathyroid A and B vaccine made by mixing three simple vaccines one from salmonella typhi and two from salmonella paratyphi. These vaccines can be further classified as monovalent or polyvalent if they are made from one strain of one species like yellow fever vaccine made of 17D strain of yellow fever virus and polyvalent when they are made of more than one strain for example cholera vaccine is made from two strains of vibrio cholera , Inaba and Ogawa.

A) Killed bacterial suspensions

Suspension of killed micro-organisms can be prepared by destroying with chemicals, heat, or radiation. The method of preparation includes carefully identifying the microbial strain and then inoculation onto a solid medium or suitable liquid medium under optimum conditions for a period of one to three days. The cells are then removed, washed with sterile saline and centrifuged to remove media and re-suspended in saline. The bacteria may be killed by heat, the lower temperatures are essential for avoiding damage to antigens. Generally 56℃ for one hour is done. Secondly, the microorganisms are killed chemically if heat affects antigenicity (in case of plague vaccine). Formalin approx 5% is used for plague and pertussis and phenol is used for cholera and thiomersal is used for pertussis and 75% ethanol for TAB and TABC.

Standardisation of suspension is done to determine the number of cells per ml by Helber cell or by opacity method in Browns tube.

Official killed bacterial vaccines are Cholera vaccine, plague vaccine and TAB and TABC vaccine. Cholera is a serious intestinal infection caused by bacteria vibrio cholera leading to severe diarhoea. The vaccine is used for travellers in tropical countries like India. The protection is shortlived only for six months.

Typhoid-paratyphoid vaccine or TAB vaccine- TAB vaccine is a combined vaccine used to produce immunity against the diseases typhoid, paratyphoid A, and paratyphoid B. Typhoid fever is an acute generalized fever caused by salmonella typhi and paratyphoid fever is caused by salmonella paratyphi.

Preparation- The smooth strain of salmonella typhi and salmonella paratyphi A and salmonella paratyphi B are cumulatively used to produce antigen containing TAB vaccine. The bacteria are either destroyed by heat, and phenol is used as preservative in the final preparation, or the organisms are killed by exposure to 75 % ethanol and preserved by 25 % ethanol. The resulting vaccine is preserved with 22.5% ethanol and the potency of vaccine is found to be double in heat treated vaccine. Storage conditions of these vaccines are at 2 to 4℃ without freezing.

B) Living bacterial suspensions

Suspension of non-activated live micro-organisms is also called attenuated micro-organisms. Preparation of dead vaccine is not always feasible as some times antigens are damaged. Attenuation of micro organisms is done to weaken the antigen so that it is safe but still able to stimulate antibody production. Although most attenuated vaccines are viral, some are bacterial in nature. Attenuated vaccines have some advantages and disadvantages. They typically provoke more durable immunological responses and are the preferred type for healthy adults. But they may not be safe to be used in immunocompromised individuals.

BCG Vaccine - Bacillus Calmette–Guerin (BCG) vaccine is a vaccine primarily used against tuberculosis. It is a suspension of living cells of strain of Mycobacterium tuberculosis known as the bacillus of Calmette and Guerin. The French bacteriologists Calmette and Guerin investigated attenuated rather than dead vaccines. In countries where tuberculosis or leprosy is common, one dose is recommended in healthy babies as close to the time of birth as possible.

Preparation: BCG is prepared from a strain of the attenuated (virulence-reduced) live bovine tuberculosis bacillus, Mycobacterium bovis that has lost its ability to cause disease in humans specially subcultured in a culture medium. Because the living bacilli evolve to make the best use of available nutrients, they become less well-adapted to human blood and can no longer induce disease when introduced into a human host. Still, they are similar enough to their wild ancestors to provide some degree of immunity against human tuberculosis. Before use, the bacterial strain is rigorously checked for antigenicity and freedom from contamination. It is grown on a liquid medium for not more than 14 days. Then, the antigens are separated by centrifugation, washed and suspended in vehicle. The liquid vaccine is replaced by freeze dried form because the liquid vaccine rapidly deteriorates even when stored at 2 to 10℃ become unfit after 14 days due to loss of viability. It has short life duration while freeze dried preparation stored under same conditions

retains its potency for at least a year. BCG tends to get clumpy when grown in conventional media, to overcome this, a nonionic surfactant polyoxyethylene ether is added in medium. The BCG vaccine can be anywhere from 0 to 80% effective in preventing tuberculosis for a duration of 15 years; however, its protective effect appears to vary according to geography and the lab in which the vaccine strain was grown. The Bacillus Calmette–Guerin (BCG) vaccine offers a variable amount of protection against leprosy in addition to its target of tuberculosis. This is known as BCG killed mycobacterium leprae vaccine rather than BCG vaccine alone.

Anthrax vaccine- Vaccines against the livestock and human disease anthrax—caused by the bacterium bacillus anthracis have a prominent place in the history of medicine, from Pasteur's pioneering 19th-century work with cattle (the first effective bacterial vaccine and the second effective vaccine ever) to the controversial late 20th century use of a modern product to protect American troops. Human anthrax vaccines were developed in the late 1930s. The current vaccine approved by the U.S. Food and Drug Administration (FDA) was formulated in the 1960s.Currently administered human anthrax vaccines include acellular and live spore varieties. All currently used anthrax vaccines show considerable local and general reactogenicity (erythema, induration, soreness, fever) and serious adverse reactions occur in about 1% of recipients.New third-generation vaccines being researched include recombinant live vaccines and recombinant sub-unit vaccines of anthrax.

Preparation- Killed bacterial preparation is usually employed in the preparation of anthrax vaccine. After that a number of experimental anthrax vaccines are undergoing pre-clinical testing, notably the bacillus anthracis protective antigenwith various adjuvants such as aluminium hydroxide (Alhydrogel), saponin QS-21, and monophosphoryl lipid A (MPL) in squalene/lecithin/Tween 80 emulsion (SLT). One dose of each formulation has provided significant protection (> 90%) against inhalational anthrax in.

3. Viral Suspensions

The immunity gained after recovery from viral diseases is often lifelong for example after mumps, measles, smallpox and yellow fever. The long lasting immunity is contributed by long incubation periods and transportation to all parts of reticulo-endothelial system, providing a

strong and continuous antigenic stimulus to the body. Some viral infections like cold and influenza gives short lived immunity because virus are confined to epithelial cells of respiratory tract and carried from one cell to other by mucus therefore antigenic stimulus is much weaker.

Many of these are active viruses that have been cultivated under conditions that disable their virulent properties, or that use closely related but less dangerous organisms to produce a broad immune response. Examples of viral vaccines include the viral diseases yellow fever, measles, mumps and rubella and the bacterial disease typhoid. The attenuated viral vaccines are more advantageous than the inactivated (dead) vaccines, because immunity is strong and long lasting as living virus multiplies in tissues, due to this smaller doses are effective. Also labile antigens are destroyed by inactivation process. But, attenuation is more difficult than inactivation.

As viruses are intracellular parasites they grow only within living cells in living animals, fertile eggs or tissue cultures. Nowadays very less vaccine is made from viruses grown in living animals because it is inconvenient and costly. Many viruses are grown in fertile eggs (chick embryo) as it gives products free from contamination, although a strict aseptic technique must be maintained throughout the process. Different viruses grow better in particular parts of embryo for example vaccinia in chorioallantoic membrane, influenza in allantoic fluid, yellow fever in embryo and typhus rickettsia in yolk sac.

Inoculation on chorioallantoic membrane

Eggs are incubated for twelve days and are candled to mark a triangle at well developed choriollantoic membrane at air sac. The triangle is lifted gently to separate it from shell membrane and a drop of sterile saline is pipette on the membrane, consequently chorioallantoic membrane falls away from shell membrane. The virus is then inoculated through the opening and site is covered with hard and soft paraffin and covering the opening with adhesive tape. The eggs are incubated while keeping the inoculation site uppermost.

Inoculation into embryo: eggs incubated for seven to eight days are used and virus is seeded through a hole over the air sac, directly into the embryo by syringe. Inoculation into yolk sac is also done and also in allantoic fluid is done after 10 to 13 days of incubation similar to the abovementioned method. Tissue culture is now a major virus vaccine production method.

1. Small pox vaccine - Smallpox vaccine, the first successful vaccine to be developed, was introduced by Edward Jenner in 1796. He followed up his observation that milkmaids who had previously caught cowpox did not later catch smallpox by showing that inoculated cowpox protected against inoculated smallpox. The vaccine is made from a virus called vaccinia, which is a poxvirus similar to smallpox, but less harmful. The smallpox vaccine contains live vaccinia virus, not a killed or weakened virus like many other vaccines.

   Smallpox was the first human infection to be successfully eradicated by vaccination. By 1977, smallpox, the most feared and devastating of all infectious diseases, was eliminated from the face of the earth. The last case of natural smallpox occurred in Somalia. Success in eradicating smallpox has provided hope for eradication of other devastating diseases, such as polio, rubella and measles.

   Preparation: The vaccine is obtained from lesions produced on skin of suitable living mammals for example sheep or calves. Healthy calves or sheeps are quarantined and thoroughly examined. The flanks and abdomen are scrubbed, disinfected shaved and scratched and inoculated by rubbing a seed of virus into scratches, under strict aseptic condition. After four to five days vesicles containing virus are developed. The animals are killed, the contents of vesicles are removed, the pooled material is homogenized and purified by grinding with equal volume of glycerol and storing for long period at -10℃. These days phenol in 0.4 % concentration is also used and incubated at 22℃ for 2 days for purification. Further tests are performed to confirm absence of pathogens.

   Alternatively vaccine can be prepared by viruses grown in fertile hen eggs. The general method of preparation in fertile eggs include theremoval of thickened and infected chorioallantoic membrane after incubation of eggs for three days. The membrane is separated into saline and frozen and diluted with saline similar to vaccine from free living animals.

   Tissue culture methods are also developed using calf embryo skin and chick embryo cells.

   Liquid small pox vaccine retains its potency for a year at -10℃. Storafe life at 2 to 10 ℃ is two weeks. The freezedried product is more stable indefinitely below 10 ℃ and for a year at 22℃.

4. Rabies vaccine- Rabies vaccine is a vaccine used to prevent rabies.There are a number of vaccines available that are both safe and effective. They can be used to prevent rabies before and for a period of time after exposure to the virus such as by a dog or bat bite. The immunity that

develops is long lasting after a full course. The first rabies vaccine was developed by Louis Pasteur which was a greatest achievement in immunization. Pasteur first showed that the virulence of virus obtained from saliva of mad dogs can be enhanced by passage through series of several rabbits, eventually it becomes stable virus and its incubation time is shortened from 60 to 6 days. Further, it was found by him that the virus can be attenuated by drying the spinal cords of rabbits. By starting immunization with an emulsion of highly attenuated spinal cord dried for 14 days, he was able to prevent development of rabies in animals and humans. Protection after infection is possible because of long incubation period of 60 days for leg bite and 30 days for bite in head region. Over the years the method given by Pasteur is modified, rabbit spinal cords are replaced by rabbit brains. And further attenuation is done by chemicals.

As per pharmacopoeia method, the rabbits or sheep are injected intracerebrally with fixed rabies virus. After symptoms for 24 hours, they are paralysed and killed; brains are harvested and homogenized in sodium chloride injection. The viruses are inactivated by phenol or formaldehyde. The vaccine prepared by brains and nervous tissue may cause serious nervous complications as an allergic response, to avoid them alternatively vaccines are prepared in fertile hen eggs.

Doses are usually given by injection into the skin or muscle. After exposure vaccination is typically used along with rabies immunoglobulin. It is recommended that those who are at high risk of exposure be vaccinated before potential exposure. Vaccines are effective in humans and other animals. Vaccinating dogs is very effective in preventing the spread of rabies to humans.

Rabies vaccines may be safely used in all age groups. About 35 to 45 percent of people develop a brief period of redness and pain at the injection site.About 5 to 15 percent of people may have fever, headaches, or nausea. After exposure to rabies there is no contraindication to its use. Most vaccines do not contain thimerosal. Vaccines made from nerve tissue are used in a few countries, mainly in Asia and Latin America, but are less effective and have greater side effects. Their use is thus not recommended by the World Health Organization. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system.

3. Poliomyelitis vaccine

Poliomyelitis is a disease which can cause paralysis of limbs therefore it is more feared, although 90 % of infections are mild and cause no symptoms only 1 percent cause paralysis. The virus first invades the oropharyngeal and intestinal mucosa cells, remain there, produce long lasting immunity and removed. While in some individuals virus can reach to nervous system through blood stream via lymphatics from intestinal mucosa leading to paralysis.

The first successful prophylactic vaccine was parenteral inactivated vaccine which stimulates production of antibodies in blood, an inactivated poliovirus given by injection (IPV) and a weakened poliovirus given by mouth (OPV). The second type of vaccine contain attenuated microorganism given orally which invade by normal route producing local antibodies in alimentary canal. Live oral vaccine is more preferred in practice.

Oral Polio Vaccine (OPV) Attenuated or Sabin immunization

OPV is a mixture of live attenuated poliovirus strains of each three serotypes. The action of oral polio vaccine (OPV) is two-pronged. OPV produces antibodies in the blood ('humoral' or serum immunity) to all three types of poliovirus, and in the event of infection, this protects the individual against polio paralysis by preventing the spread of poliovirus to the nervous system. The polyvalency of vaccine gives long lasting immunity. The three types of virus are grown in suspended cell cultures or fixed cell cultures of monkey kidney tissues. Rhesus monkeys are quarantined and checked for tuberculosis and other infections. OPV is an attenuated vaccine, produced by the passage of the virus through nonhuman cells at a sub-physiological temperature, which produces spontaneous mutations in the viral genome. Oral polio vaccines were developed by several groups, one of which was led by Albert Sabin. The various vaccines were carefully evaluated for their ability to induce immunity to polio, while retaining a low incidence of neuropathogenicity in monkeys. Fifty-seven nucleotide substitutions distinguish the attenuated Sabin 1 strain from its virulent parent (the Mahoney serotype), two nucleotide substitutions attenuate the Sabin 2 strain, and 10 substitutions are involved in attenuating the Sabin 3 strain. The primary attenuating factor common to all three Sabin vaccines is a mutation located in the virus's internal ribosome entry site, which alters stem-loop structures and reduces the ability of poliovirus to translate its RNA template within the host cell. The attenuated poliovirus in the Sabin vaccine replicates very efficiently in the gut, the primary site of infection and replication, but is unable to replicate efficiently within nervous system tissue. OPV is usually provided in

vials containing 10–20 doses of vaccine. A single dose of oral polio vaccine (usually two drops) contains 1,000,000 infectious units of Sabin 1 (effective against PV1), 100,000 infectious units of the Sabin 2 strain, and 600,000 infectious units of Sabin 3. The vaccine contains small traces of antibiotics—neomycin and streptomycin—but does not contain preservatives. Oral polio vaccines cause about three cases of vaccine-associated paralytic poliomyelitis per million doses given. This compares with 5,000 cases per million who are paralysed following a polio infection. Both are generally safe to give during pregnancy and in those who have HIV/AIDS but are otherwise well.

Inactivated parentaral vaccine

The Salk vaccine, IPV, is based on three wild, virulent reference strains, Mahoney (type 1 poliovirus), MEF-1 (type 2 poliovirus), and Saukett (type 3 poliovirus), grown in a type of monkey kidney tissue culture (Vero cell line), which are then inactivated with formalin. The injected Salk vaccine confers IgG-mediated immunity in the bloodstream, which prevents polio infection from progressing to viremia and protects the motor neurons, thus eliminating the risk of bulbar polio and post-polio syndrome. The World Health Organization (WHO) recommends all children be fully vaccinated against polio. The two vaccines have eliminated polio from most of the world, and reduced the number of cases reported each year.

4. MMR vaccine - The MMR vaccine is a vaccine against measles, mumps, and rubella (German measles). The first dose is generally given to children around 9 to 15 months of age, with a second dose at 15 months to 6 years of age, with at least 4 weeks between the doses.

   Preparation- The various steps of preparation of MMR vaccine are as follows-

   a. The viruses are grown aseptically in the primary culture of chick embryo cells or other suitable living cellular structure.
   b. In these chick embryo cells, embryo is derived from healthy flock free from avian.
   c. The viral suspensions are harvested at a time suitable for viral culture and tested for identity and sterility.
   d. The harvested viruses are pooled with each other and a suitable stabilizer is added to the clarified vaccine.

Keeping in view the tremendous and geometrical advancement in vaccination approaches about safety, longevity, efficacy following vaccines have developed and delivered for mass protection as well as specific applications :-

Synthetic peptide vaccines – A synthetic vaccine is a vaccine consisting mainly of synthetic peptides, carbohydrates, or antigens. Synthetic peptides are short amino acid chains containing only the neutralizing epitope of an immunogenic protein from a disease agent. They are usually considered to be safer than vaccines from bacterial cultures. Creating vaccines synthetically has the ability to increase the speed of production. The production of inactivated/killed vaccines essentially involved the live and pathogenic forms of the parasites of disease. The chemically synthesised peptide obtained in purest form having almost little contamination and known as synthetic vaccine.

Preparation-A plasmid or a virus or a part of standard molecule of DNA is selected as carrier. Gene is subsequently added as carrier molecule model that is essentially encodes the peptide of interest. Resultant recombinant molecule is replicated in host environment and enhance it's multiplication. For commercial production of this desired peptide grow fermenters are used.

Multivaccine System- It represents new era of vaccination that is based on r DNA or recombinant DNA technology. Scientific data show that getting several vaccines at the same time does not cause any chronic health problems. A number of studies have been done to look at the effects of giving various combinations of vaccines, and when every new vaccine is licensed, it has been tested along with the vaccines already recommended for a particular aged child. The recommended vaccines have been shown to be as effective in combination as they are individually. Sometimes, certain combinations of vaccines given together can cause fever, and occasionally febrile seizures; these are temporary and do not cause any lasting damage. Based on this information, both the Advisory Committee on Immunization Practices and the American Academy of Pediatrics recommend getting all routine childhood vaccines on time. Examples are DPT vaccine, human birth control vaccine (HCG) is being developed by national institute of immunology, New Delhi.

Newer advanced vaccines for cancer, birth control vaccine and AIDS vaccine are being explored by recombinant DNA technology. Future vaccines include vaccines for Alzheimer's , meningitis and vaccine against cervical cancer .

4. Antibody containing preparations-SERA

The plasma of immunized person or animal contains a large number of antibodies which are found in serum after its separation when blood is allowed to clot. A serum may contain antitoxic, antibacterial or antiviral antibodies and therefore it may be called antitoxin, antibacterial or antiviral serum, collectively they are known as Antisera. Most antisera are obtained from animals where active immunity is stimulated artificially but for some diseases like measles, human serum derived products are used. The general methods of preparation of the official antitoxins are essentially similar.

Antitoxin

1. Diphtheria Antitoxin

For diphtheria and staphylococcus antitoxins, the horses are preferred because large volumes of blood can be withdrawn without ill effects and also their red blood cells settle quickly and pack tightly, which facilitates easy separation of serum. Occasionally goats are used for individuals sensitive to horse proteins. First of all horses are isolated for seven days for initial examination of infections and they are immunized against tetanus. After isolation the increasing amounts of toxoid are injected into neck muscles every few days for several months. Starting dose may be 5 ml to reach upto 600 ml until the satisfactory antibody titre is obtained. Afterwards 8 litres of blood is withdrawn from jugular vein in the bottles containing anticoagulant. The bleeding is repeated twice over next 8 days and then given 10 days rest. Afterwards short course of antigen is repeated.

Refinement of serum is done as horse serum contains high concentration of proteins. Sometimes hypersensitive response like anaphylactic shock and serum sickness develops in some patients leading to risk. The refinement is done by fractional precipitation and proteolytic digestion so that only active fragments of antitoxin are separated and other undesirable proteins are digested and denatured by addition of ammonium sulphate.

Botulinum antitoxin

Botulism occurs due to exotoxin of Clostridium botulinum. It results from eating contaminated foods that contain toxin released by anaerobic bacteria. The toxin is extremely dangerous. Polyvalent antitoxin is prepared containing mixture of antitoxins from all the three strains A, B and E of clostridium botulinum.

Gas Gangrene antitoxin

Gas gangrene is a deep wound infection with dirt caused by anaerobic exotoxin producing bacilli- Clostridium welchii, Cl. Septicum, Cl. Oedematiens. This infection is also known as septique. The most potent toxin produced by each species is alpha-toxin and it must be present in the antigen used for immunizing the horse. Mixed antitoxin from all the three strains is preferred, this antitoxin is used for prophylaxis after serious sports injury and road injuries, but antibiotics are increasingly preferred.

Tetanus antitoxin

The antitoxin for treatment of tetanus is of no value if it is administered after the symptoms have developed, because once the toxin from clostridium tetani combines with nerve cells can not be reversed back. For many years it has been used as prophylactic in wars and accidents having deep penetrating uncleaned wounds. Generally immunization of schoolchildren and kids is done with tetanus vaccine instead these days.

Antibacterial Sera

Antibacterial sera provide passive immunity to disease caused by endotoxin producing bacteria. In past antisera against pneumonia, meningitis and typhoid were used but these are now replaced by chemotherapy.

Antiviral Sera

The viruses are intracellular parasites and antibodies cannot penetrate cells, the inactivation of virus must take place in body fluids and on surfaces of invasion. Human serum is used as source of antiviral serums because horse is not susceptible to several infections to which human are susceptible but rabies antiserum is prepared in horses.

Rabies antiserum

A course of dead rabies virus is given to horse, after development of good immunity, it is replaced by living virus. The antibodies are collected and purified and refined by protein

fractionation as mentioned earlier. The gamma globulin fractioncontaining antiviral antibodies is separated. The rabies antitoxin in conjuction with rabies vaccine is administered to patient who have bitten by a rabid animal, within 24 hours of exposure to infection.

Human Normal Immunoglobulin Injection

Human normal immunoglobulin injection is also called human gamma globulin injection or simple gamma globulin. It contains all of gamma G globulins of human plasma. Gamma globulin consist of three components, IgG, IgM, IgA antibodies. IgG antibodies constitute 90% of total immunoglobulins and are produced by prolonged and strong antigen stimuli. It is obtained by fractionation of human plasma proteins. They are used against serious viral infections like measles Rubella and infectious hepatitis.

a) Storage and stability of vaccines

Potency of immunological preparation can be preserved by preventing denaturation of proteins (antigens or antibodies) or maintaining viability of young cells. Preservative bactericide cannot be used in living bacterial suspensions like BCG vaccine. Also, they are not used for living viral vaccines. Bactericides can be added to dead bacterial and viral vaccines, toxoids and sera. Phenol and Cresol are preferred, 0.01 to 0.02 percent of thiomersal is satisfactory. The storage at lower temperatures are essential as loss of viability and denaturation is caused by temperature. The optimum storage temperature for most products is just above freezing point, generally 2-4℃. Storage between 2 and 10 ℃ is directed for most vaccines. Most of viral vaccines are stable at or below freezing points, while bacterial vaccines deteriorate if allowed to freeze. For example poliomyelitis oral vaccine can be stored at frozen state can be have shelf life upto 2 years. While at 20 ℃ have 7 days shelf life. Small pox vaccine at – 10 ℃ have shelf life of 1 year while at 2 to 10 ℃ have shelf life of 14 days. Yellow fever vaccine stored at 0 ℃ has shelf life of 1 year while if it is stored at room temperature it have shelf life of few days. Freeze dried vaccines are comparatively stable than the corresponding liquids. For example freeze dried small pox vaccine must be stored below 10℃ and at 0 ℃ approx. Protection of light is also essential to prevent decomposition but light resistant containers are not used. Storage in a carton is satisfactory.

Schedule of vaccination for Child

The schedule of immunization for child recommended by Indian Academy of Pediatrics and Ministry of health, Govt of India, National schedule of immunization is as follows:

Hepatitis B – at time of birth as early as possible.

Name of vaccine	Schedule Time	Dose	Route and site
Hepatitis B	At the time of birth as early as possible within 24 hours	0.5 ml	Intramuscular (antero-lateral side of mid Thigh)
BCG vaccine	At the time of birth as early as possible within one year	0.05- 0.1 ml	Left upper arm
Oral Polio Vaccine (OPV)-0	At the time of birth within 15 days	2 drops	Oral
OPV-1, 2, 3	6 weeks, 10 weeks, 14 weeks (Till 5 years Age)	2 drops	Oral
IPV (Inactivated Polio vaccine) 1 &2	6 weeks and 14 weeks (1 year of age)	Intra-dermal	Upper arm
Pentavalent vaccine-1,2,3 (diphtheria, Pertussis/whooping cough and tetanus), Hepatitis B and Hib vaccines	6, 10, 14 weeks (1 year of age)	0.5 ml	Intra muscular (Antero-lateral side of mid thigh)
Rotavirus vaccine 1, 2 3	6, 10, 14 weeks (1 year of age)	5 drops	Oral
PCV (Pneumococcal vaccine) 1, 2 and booster	6 , 14 weeks and 9 months (upto 1 year of age)	0.5 ml	Intra muscular (Antero-lateral side of mid thigh)

MCV/ MMR (Mumps,Measles Rubella)	On completed 9 months upto 5 years	0.5 ml subcutaneous	Right Upper arm
Japanese encephalitis	On 9 completed months upto 15 years	0.5 ml subcutaneous	Left upper arm