FISH VACCINE

The process of stimulating protective immune responses in fish against pathogenic microorganisms by exposing them to non-pathogenic forms or components of microorganisms is referred to as **vaccination.**

When feasible, effective and safe, vaccination is one of the most cost-effective measures for controlling infectious disease, not only in companion animals but also in food-producing animals.

- Although fish immune systems are primitive compared with mammals, there seem to be more similarities than differences.
- In the limited number of fish species studied, the major antibody type is an immunoglobulin (Ig)M-like isotype that typically exists as a tetramer in its secreted form
- No isotypes corresponding to mammalian IgG, IgA or IgE have been identified in fish and
- The secondary humoral immune response in fish is, if present at all, less prominent than in mammals.

- Lack of detailed knowledge of the immune systems in different fish species limits the possibilities to study both pathogen and vaccine-induced immunity.
- Cost effectiveness in the field is an essential limitation to commercial vaccine development.
- Fish generally need a large antigen dose compared with terrestrial animals and cost-effective inactivated viral vaccines have proven difficult to develop.
- Some species are too vulnerable to handle the stress induced during the vaccination or may develop severe side effects post vaccination.

- Yet, in other species, the major disease problems may appear in the larval or fry stages, before the animal is large enough to be vaccinated or have even developed a functional immune system.

- The apparent lack of maternal immunity in fish also limits the possibilities to protect offspring by parental vaccination.

Vaccination is one of the important means of controlling disease.

In 1798, Edward Jenner worked on small pox. He employed the term ‘vaccine’ (vaccination for protective inoculation).

Pasteur extended Jenner’s findings to other infective diseases such as anthrax, rabies and chicken cholera.

By ‘vaccination’ it is possible to induce active immunity to diseases.

Immunisation is brought about by the use of killed or weakened (attenuated) bacteria.

The immune system recognizes and begins to produce antibodies.

Control of diseases by vaccination has a number of advantages over chemotherapeutic methods.

Vaccination is preventive measure.

The use of vaccines has entered in the field of aquaculture recently.

Because of the intensive culture systems, many industries have resorted to the routine use of vaccines which confer a high degree of protection when correctly used.

Use in salmon, trout, Mediterranean sea bass and even in shrimp and lobsters is now a standard part of husbandry North and South America and Asia.

The concept of vaccinating fish on a commercial scale has now been realized with respect to Enteric Red Mouth and Vibriosis.

Fish immunization began in 1942 by Duff, with the successful oral immunization of trout against bacterium *Aeromonas salmonicida*.

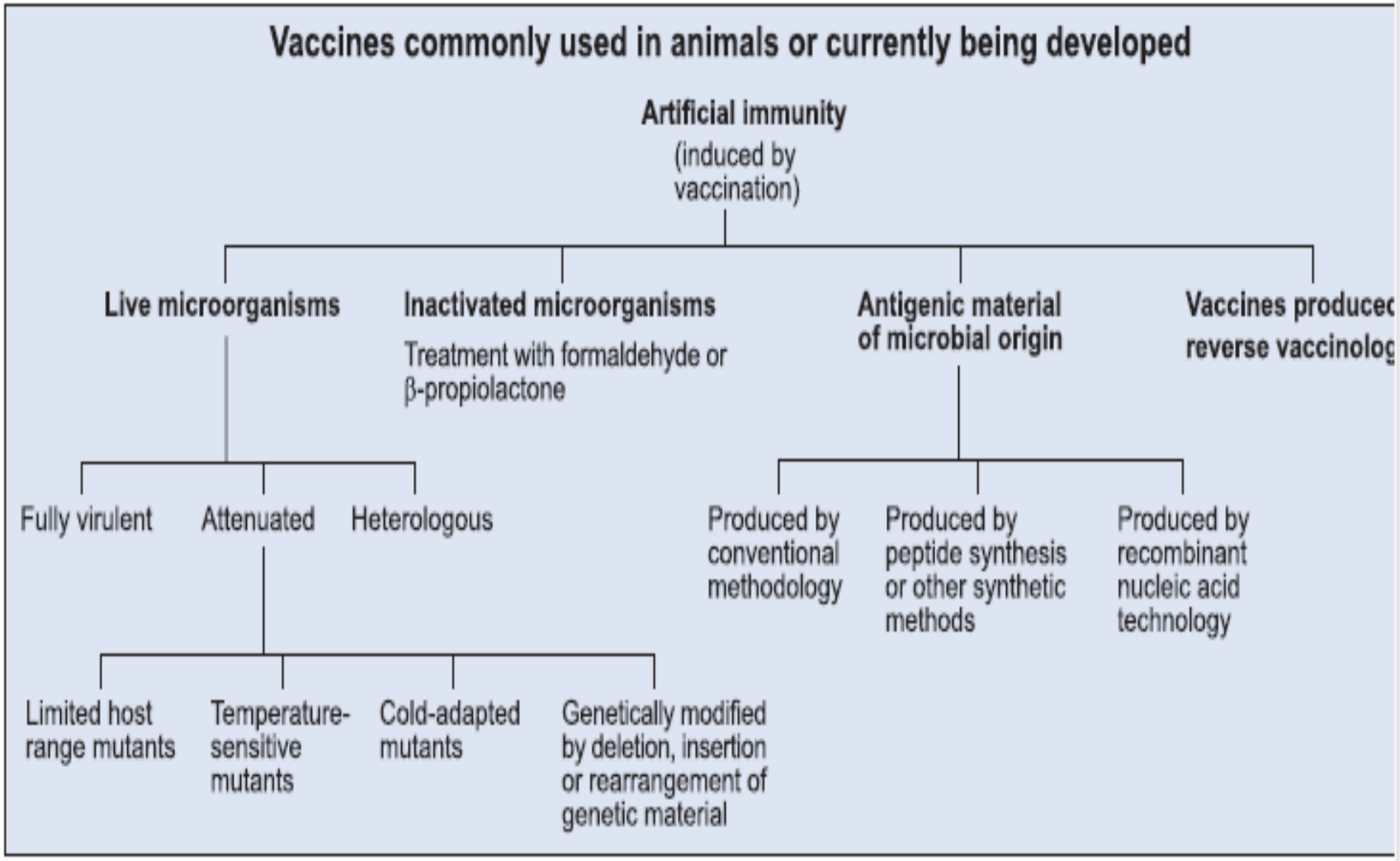
Fish vaccines in general, fall into three major categories, namely, killed whole cell vaccine, live-attenuated vaccine and recombinant DNA based vaccines.

Efficacy of these vaccines has been improved using adjuvants, immunostimulants or vaccines carriers.

However, it is still affected by the routes of vaccine administration.

In general, injection is better than immersion and oral administration.

Vaccines


Vaccines commonly used in animals or currently being developed
Artificial immunity
(induced by vaccination)
Live microorganisms
Inactivated microorganisms
Treatment with formaldehyde or β-propiolactone
Antigenic material of microbial origin
Vaccines produced
reverse vaccinolog
Fully virulent
Attenuated
Heterologous
Produced by conventional methodology
Produced by peptide synthesis or other synthetic methods
Produced by recombinant nucleic acid technology
Limited host range mutants
Temperature-sensitive mutants
Cold-adapted mutants
Genetically modified by deletion, insertion or rearrangement of genetic material

Killed whole cell vaccines

- Killed whole cell vaccine is a suspension of heat or chemical killed pathogens that are able to induce specific protective immune response against those pathogens when administered into the host.
- They are used in controlling fish bacterial pathogens such as, *V. anguillarum*, *V. salmonicida*, *V. ordalli*, *Y. ruckeri*, and *A. salmonicida*.
- These killed vaccines are formalin inactivated whole cell vaccines administered with or without adjuvants and are commercially available.
- These bacterial vaccines are highly immune protective, and are cheap to produce, but are not known at present as to what specific antigens of these vaccines are involved in offering protection.

- In many cases it is believed that the protective substances are lipo polysaccharides.
- Killed vaccines have been developed for some pathogenic fish viruses such as infectious pancreatic necrosis virus (IPNV), infectious haematopoietic necrosis virus (IHNV), viral haemorrohagic septicaemia virus (VHSV) and spring viremia of carp virus (SVCV).
- Injection of rainbow trout fry with the inactivated IPNV offers good protection in rainbow trout but when administered in brook trout with Freund's complete adjuvant it induces strong humoral response with poor protection.
- Successful use of killed VHSV in rainbow trout has also been recorded.

- Formalin-inactivated IHNV has been found to protect rainbow trout against lethal IHNV when immunized at high concentration.
- Although all these above vaccines look promising at laboratory scale none of them has been commercialized.
- It is only the killed vaccine of spring viremia of carp virus (SVCV) that was commercially available for some years. This vaccine comprises of two inactivated strains of SVCV emulsified in oil.

Disadvantages of using killed virus vaccines

- High cost of their production in cell culture, cumbersome method of purification and delivery.
- In general, killed vaccines alone trigger only the humoral immune response and not the cell-mediated immune response.
- Further, induces protective immunity, which fades away over time and needs to be given in booster doses.

2.Live –attenuated vaccines

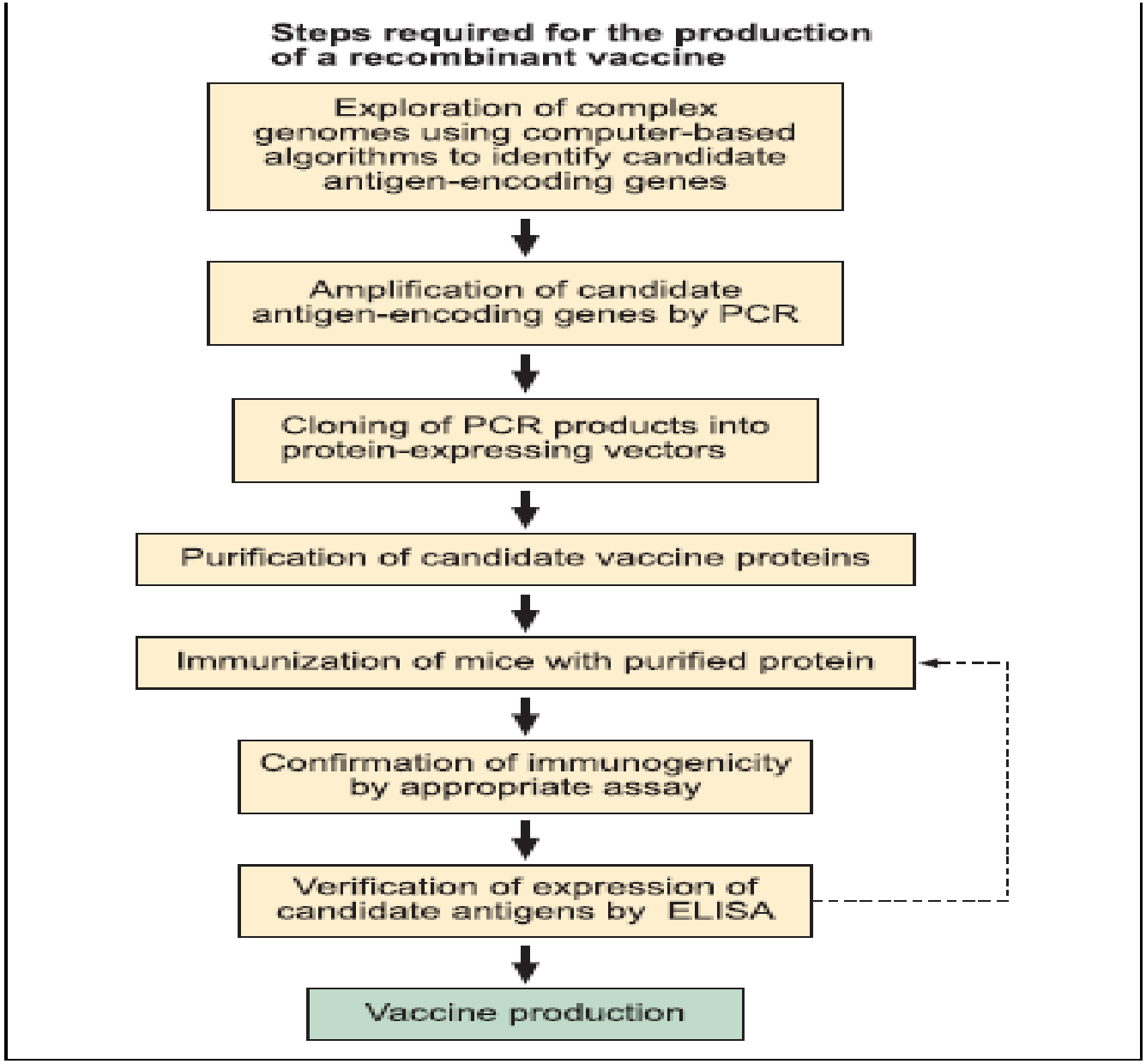
- It is a suspension of attenuated live pathogens that are able to replicate inside the host and induce protective immune response but unable to cause disease.
- They mimic the actual infection by pathogens and hence a small dose of vaccine is enough to induce long lasting protective immune response.
- Can induce both humoral and cell-mediated immune responses.
- These are strong stimulants of cell-mediated immune response. These preferentially enhance T cell proliferative response relative to B cell responses.
- Some of the conventional live viral vaccines have been produced against VHSV,IHSV and IPNV.

- Avirulent strains of IHNV are also used as live vaccines.
- Use of VHSV-attenuated strains obtained through serial passage of VHSV in carp-cell line under progressive increase of temperature has been used as live vaccine.
- Protection of goldfish against some common ectoparasites has been observed by intraperitoneal and immersion immunizations with live tomites of *Ichthyophthirius multifiliis* and *Tetrahymena pyriformis*.

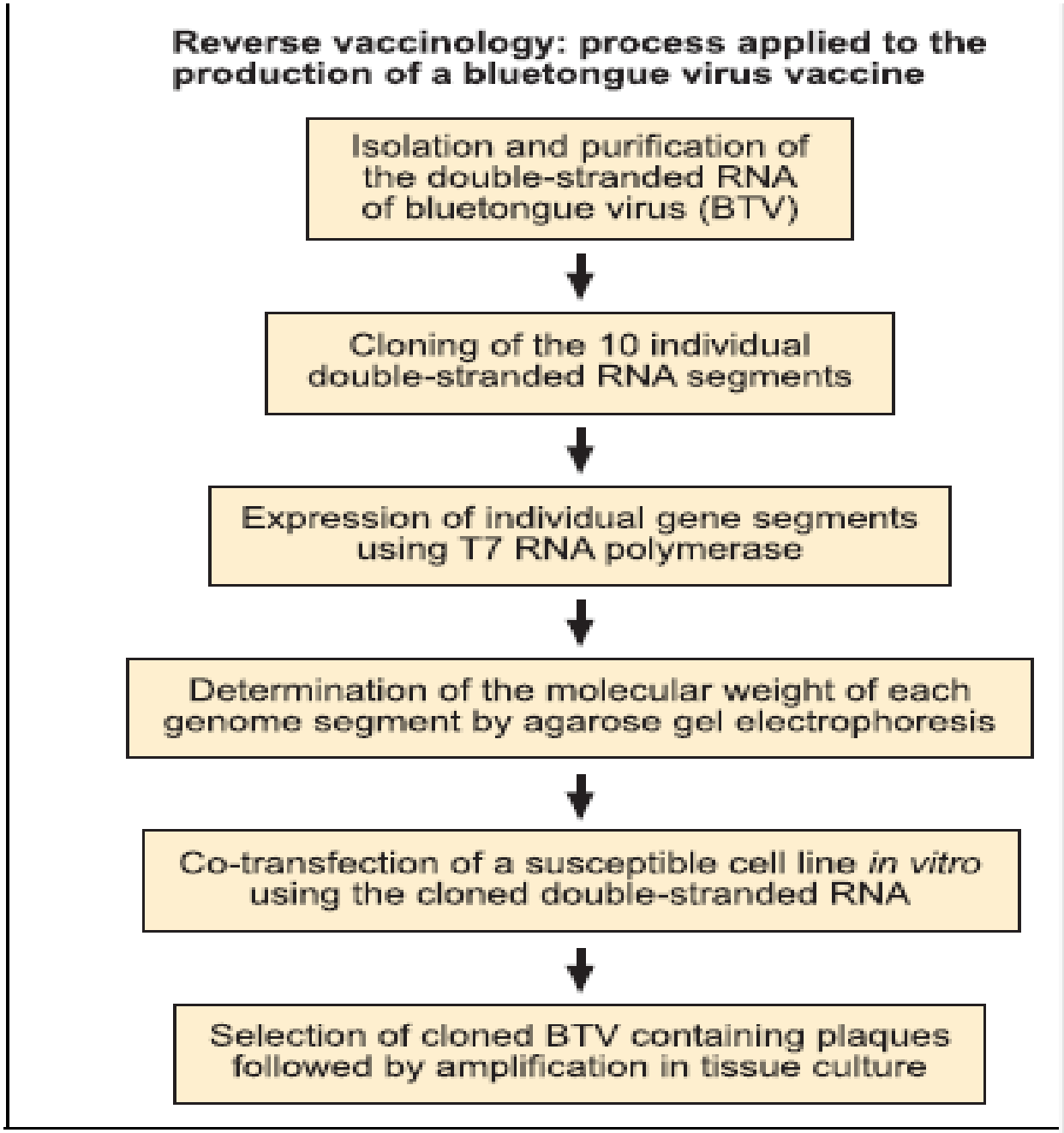
- Although some of these vaccines are found useful as live vaccines in laboratory, so far none of them has been licensed for field trial.
- This is because of some of the possible disadvantages,
- Such vaccine strains becoming virulent in non-target species,
- Possibility of reversion to pathogenic state and problems associated with residual virulence.

3. Recombinant DNA-based vaccines

- R DNA technology has been widely used in development of novel vaccines that are now collectively termed as ‘recombinant DNA-based vaccines’ or ‘new generation vaccines’.
- Different types of vaccines based on recombinant DNA technology have been developed which include:
- Recombinant immunogenic protein vaccines or epitopes purified from vectors carrying the gene of interest produced in prokaryotic or eukaryotic expression systems,peptide vaccines,
- live vaccines produced by defined genetic manipulations and microbial vectors carrying gene coding for immunogenic protein and DNA vaccines.


Steps required for the production of a recombinant vaccine
Exploration of complex genomes using computer-based algorithms to identify candidate antigen-encoding genes
Amplification of candidate antigen-encoding genes by PCR
Cloning of PCR products into protein–expressing vectors
Purification of candidate vaccine proteins
Immunization of mice with purified protein
Confirmation of immunogenicity by appropriate assay
Verification of expression of candidate antigens by ELISA
Vaccine production

Reverse vaccinology: process applied to the production of a bluetongue virus vaccine



1. Recombinant protein vaccines

- Production of a recombinant protein vaccine starts with identification of the immunogenic subunit or protein from the pathogen of interest and verification of its immunogenicity *in vivo* and *in vitro*.
- For example, purified glycoproteins from IHNV and VHSV have been used as subunit vaccines in fish and shown to be immunoprotective, and used widely for recombinant vaccine production.
- Similarly, an RNA-free subunit vaccine prepared from grass carp haemorrhage virus (GCHV) treated with 1% NP40 in low salt solution has been shown to induce more than 80% protection in carp.
- Once, the immunogenic proteins or subunits of pathogen are identified, the gene(s) involved in coding for them can be introduced into a vector, over-expressed in expression hosts and can be used as recombinant protein vaccines.

- pThe vector systems used to express recombinant proteins are viruses or bacterial lasmids.
- Expression systems commonly used are prokaryotic and eukaryotic cells.
- Prokaryotic expression system comprises of bacteria such as, *Escherichia coli*, and the eukaryotic expression system comprises of yeast, insect cells and mammalian cells.

Disadvantages of prokaryotic systems

- They lack the signals required for proper post-translational modification and there lies the problem of improper folding and lack of glycosylation.
- This leads to production of proteins of unpredicted antigenicities. in the form of inclusion bodies that need to be treated biochemically before being used as vaccine.
- This biochemical treatment of denaturation and renaturation of recombinant protein reduced its immunogenicity.

Advantages of prokaryotic expression system

- High level expression of recombinant protein (often more than 30%), well studied genetic and fermentation system of *E. coli* and easy scaling up of vaccine production.

- In the case of eukaryotic expression system, although the problem of folding and glycosylation does not exist, the final yield of expressed protein remains low, and hence the scaling up of the production process is difficult.
- Both prokaryotic as well as eukaryotic expression systems have been used to produce fish viral, bacterial and parasitic antigens, and prokaryotic system is most widely used.

b) Peptide vaccines

- Peptide vaccines comprise of synthetic peptides that are able to induce protective immune response when administered into the host.
- To produce peptide vaccines it is necessary to identify immunogenic regions, also known as ‘epitopes’ on the antigenic protein.
- The term epitope refers to a stretch of 6-8 amino acids on antigens that specifically binds to antibodies or to receptors on immune T cells.
- Those epitopes that bind to the antibody produced by specific B cells are called as B-cell epitopes while those recognized by receptors on the surface of activated T-cells are termed as T-cell epitopes.
- Monoclonal antibodies are indispensable to identify the B-cell epitopes.

- A region with high sequence variability among several strains of a pathogen is also chosen as a candidate for synthetic peptide vaccine.
- Epitope mapping and use of peptide vaccines against fish pathogens are still in its infancy.
- Some of the B-cell epitopes have been identified on some fish viral proteins such as IHNV glycoprotein.
- Synthetic peptide vaccines emulsified with Freund's complete adjuvant has induced poor neutralizing antibodies than that of the native virus fish sera, which indicates that peptides alone are less immunogenic than the native protein.

c) Genetically modified live vaccines

- Pathogens with defined genetic manipulations or microbial vectors carrying the gene coding for immunogenic protein can be used as live vaccines.
- Live vaccines replicate inside the recipient host resembling the natural infection and thus induce strong immunity.
- This kind of vaccine is reported to be highly immunogenic than the non-replicating vaccine products.
- Selection of a stable non-pathogenic mutant usable as live vaccine is a complex process .
- It involves tedious procedure of growing viruses in different culture conditions or introducing targeted mutations, followed by *in vivo* and *in vitro* assays.

- Some important methods of selection of attenuated mutants are, adaptation to heterologous cell line, adaptation to elevated temperature and selection of neutralizing monoclonal antibody escape mutatnts.
- The rationale behind selection of strains adjusted to such extreme conditions is that these strains are believed to be altered genetically hence resulting alteration of their virulence.
- Nucleotide sequence analysis of such strains can confirm the position of mutation.

- Further, *in vivo* and *in vitro* analysis can reveal their phenotypic variation hence aiding in selection of such strains as candidates for live vaccine.
- Defined genetic alterations resulting in mutants with desired phenotype can be achieved using site directed mutagenesis technique also.
- Live vaccines have been used against some of the fish bacterial pathogens such as *A. salmonicida* and *A. hydrophila*.
- Several techniques such as homologous recombination, chemical mutagenesis and transposon mutagenesis are used to produce mutant bacteria those are avirulant and capable of being used as live vaccines.

d) DNA vaccines

- DNA vaccines consist of a suspension of bacterial plasmids carrying the gene coding for the immunogenic protein under the control of eukaryotic promoter.

DNA vaccines include

- an origin of replication suitable for producing high yields of plasmid in *E. coli*,
- an antibiotic-resistant gene to confer antibiotic-selected growth in *E. coli*,
- a strong enhancer/promoter and
- an mRNA transcript termination/polyadenylation sequence for directing expression in mammalian cells.
- The plasmids hence constructed are grown in *E. coli*, purified and suspended in saline and introduced into the host either by intramuscular injection or using a gene gun.

- DNA vaccines have been used in fishes with very encouraging results.
- Strong expression of reporter genes in muscle cells following intramuscular injection of plasmid constructs carrying gene of interest and reporter gene have been reported.
- When plasmids carrying luciferase gene under the control of cytomegalovirus immediate early gene promoter is injected to rainbow trout at a dose of 50µg of DNA, maximum activity is seen at 5 to 7 day post-injection and the activity of luciferase remains for 115 days.
- Combined injection of plasmids carrying VHSV and IHNV glycoprotein genes shows plasmid DNA to remain in the muscle cells up to 45 days.

- DNA immunization induced specific as well as non-specific immune response in the recipient host.
- High level of protection in clinical animal model has been observed due to the generation of specific antibodies and priming of T-cell responses.
- Significant protection of rainbow trout is observed against IHNV challenged following the injection of construct encoding the IHNV G protein.
- Apart from introducing a part of the genome of pathogen coding for immunogenic protein, it is possible to introduce a gene coding for an antibody that can target and destroy the pathogen.
- DNA vaccines overcome almost all the drawbacks of all other form of vaccines.

Advantage of DNA vaccines

- Ability to induce production of native form of protein with appropriate post-translational modifications. This has been shown in the case of DNA immunization of rainbow trout.
- Able to induce long lasting immune response and are economical and safe.
- Practical application of DNA vaccine in fish does not seem to be encouraging because most of the important fish pathogens, especially the viruses those affect fish at a very young age.
- This makes it difficult for one to administer vaccine to small fish through injection route, which is so far the only method of introducing the DNA vaccines.
- However, the injection method is useful for immunizing broodstocks of fairly large fish so as to ensure that immunity is passively transferred from mother to offspring as this being demonstrated in controlling Ich.
- It is difficult to use DNA vaccines for individual fish on a large scale in intensive aquaculture unless one can introduce DNA vaccine to fish orally or through gill filaments via aquatic medium.

Vaccine delivery system

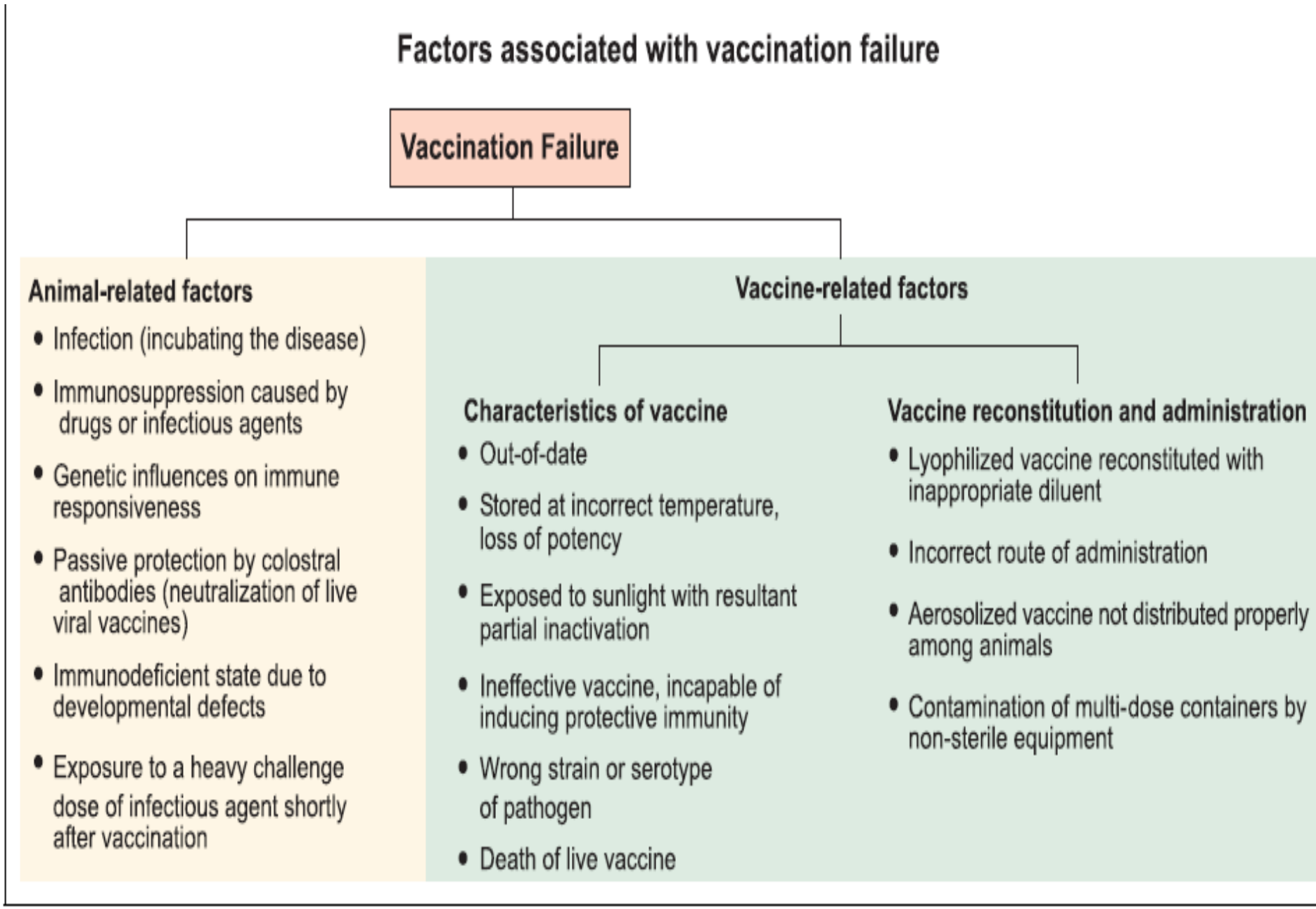
- It can be administered by injection, by immersion or by spraying directly onto the fish according to what suits an individual farm's preference.
- Small fish (1.5 to 5 gms) by direct immersion in diluted vaccine (1:10) for 30 secs.
- Larger fish (70-100 gms) sprayed with vaccine or immersion for 3-5 secs.
- Stress should be avoided at the time of handling.
- Maintain the vaccine solution at the same temperature on the holding tanks, oxygenating the vaccine solution during the vaccination procedure, etc.

Routes of administration of vaccines in fish

- The vaccines are administered to the fish in a number of ways
- Injection
- immersion
- hyper osmotic infiltration
- Bath
- spray and
- oral modes

- Oral vaccination of fish using Artemia as the vaccine delivery system can also done.
- When vaccine is given through oral route there is possibility of Ag being degraded by the digestive enzymes in the stomach.
- New approach involves first feeding the vaccine (a killed bacterial suspension) to the Artemia, and then feeding the Artemia as the first live food to the fry of the species of interest.
- It is thought that the vaccine becomes incorporated into the lipids of the Artemia and this protects it from the digestive degradation of the fish.

- Immunity in vaccinated animals tends to change with time following vaccination.
- Booster vaccination can be given.
- Duration of protection depends upon the method of vaccination, the size of the fish, their health status at the time of vaccination and the antigen used to vaccinate them.
- Vibrogen -2 vaccine is produced by Aquatic Health Limited, Greece.
- The AHL, Canada has developed another vaccine called Lipogen Triple bacterin (a combimnation furunculosis + vibriosis + hitra bacterin) to protect against furunculosis.


Factors associated with vaccination failure
Vaccination Failure
Animal-related factors
• Infection (incubating the disease)
• Immunosuppression caused by drugs or infectious agents
• Genetic influences on immune responsiveness
• Passive protection by colostral antibodies (neutralization of live viral vaccines)
• Immunodeficient state due to developmental defects
• Exposure to a heavy challenge dose of infectious agent shortly after vaccination
Vaccine-related factors
Characteristics of vaccine
• Out-of-date
• Stored at incorrect temperature, loss of potency
• Exposed to sunlight with resultant partial inactivation
• Ineffective vaccine, incapable of inducing protective immunity
• Wrong strain or serotype of pathogen
• Death of live vaccine
Vaccine reconstitution and administration
• Lyophilized vaccine reconstituted with inappropriate diluent
• Incorrect route of administration
• Aerosolized vaccine not distributed properly among animals
• Contamination of multi-dose containers by non-sterile equipment

- The first commercially available bacterial vaccines were against enteric redmouth disease (ERM, yersiniosis) and vibriosis, introduced in the USA in the late 1970s
- The first viral vaccine for fish was produced by a Czechoslovakian company (Bioveta) in 1982. The vaccine was against a carp rhabdovirus, causing spring viremia of carp (SVC) and was based on two inactivated strains of SVC virus emulsified in oil and administered by injection.
- The only commercial carp vaccine in Asia is an inactivated grass carp hemorrhage disease virus (a reovirus) vaccine, which has been widely used in China.
- A koi herpesvirus vaccine based on an attenuated strain of carp interstitial nephritis and gill necrosis virus is available in Israel.

Table 1. Major bacterial fish diseases in relation to vaccine availability.

Bacterial disease/pathogen	Major fish species affected	Primary region(s)/ country(s)	Commercially available vaccine(s)
Vibriosis (*Listonella anguillarum* and V. spp.)	Salmonids	Globally	Yes
	Cod/halibut		Yes
	Sea bass/ bream		Yes
	Amberjack/yellowtail		Yes
Coldwater vibriosis (*Vibrio salmonicida*)	Salmonids	Northern Europe, Canada/USA	Yes
Wound disease (*Moritella viscosa*)	Salmonids	Northern Europe	Yes
Furunculosis (*Aeromonas salmonicida subsp.salmonicida*)	Salmonids	Northern Europe, Canada/USA	Yes
Atypical *Aeromonas salmonicida*	Salmonids	Globally	Yes
	Various FW/SW species		No
ERM/Yersiniosis (*Yersinia ruckeri*)	Salmonids, FW	Europe, Chile, Canada/USA	Yes
Piscirickettsiosis (*Piscirickettsia salmonis*)	Salmonids	Chile	Yes
Bacterial gill disease (*Flavobacterium branchiophilum*)	Various species, e.g., salmonids and carp, FW	Canada/USA, Europe, Chile, Japan	No
Flavobacteriosis (*Flavobacterium psychrophilum*)	Salmonids, FW	Chile, Canada/USA (West)	Yes
Columnaris (*Flavobacterium columnare*)	Channel catfish	USA	Yes
	Salmonids, FW	Chile	Yes

Rainbow trout fry syndrome (*Flavobacterium psychrophilum*)	Salmonids, FW	Europe, Canada/USA, Chile	No
Enteric septicaemia of catfish (*Edwardsiella ictaluri*)	Catfish species	USA	Yes
		Asia	No
Edwardsiella septicaemia (*Edwardsiella tarda*)	Channel catfish	USA	No
	Eel, Japanese flounder	Asia	No
Bacterial kidney disease (*Renibacterium salmoninarum*)	Salmonids	Chile, Canada/USA	Yes
		Europe, Japan	No
Lactococciosis (*Lactococcus garvieae*)	Rainbow trout	Italy, France, UK	Yes
	Amberjack/yellowtail	Japan	Yes
Pasteurellosis (*Photobacterium damsela subspecies piscicida*)	Sea bream/sea bass	Mediterranean	Yes
	Amberjack/yellowtail	Japan	No
Streptococciosis (*Streptococcus iniae*)	Tilapia	Asia	Yes
(*Streptococcus phocae*)	Asian sea bass	Asia	No
	Salmonids	Chile	No

FW: Fresh water; SW: Salt water.

Table 2. Major viral diseases in fish in relation to vaccine availability.

Viral disease/pathogen	Major fish species affected	Primary region(s)/ country(s)	Commercially available vaccine(s)
Infectious pancreatic necrosis/IPNV, other aquatic birnaviruses	Salmonids Various marine species	Globally	Yes No
Pancreas disease/PDV	Salmon	UK, Ireland, Norway	Yes
Infectious salmon anemia/ISAV	Salmonids	Canada/USA (East), Norway, UK	Yes
Infectious hematopoietic necrosis/IHNV	Salmonids	Canada/USA (West)	Yes
Viral hemorrhagic septicemia/VHSV	Rainbow and brown trout, turbot, Japanese flounder	Europe, Asia	No
Viral nervous necrosis/SJNNV and several other betanodavirus	Several marine fish species, e.g., sea bass, groupers, barramundi, halibut	Globally	No
Iridoviral disease/RSIV	Red sea bream, amberjack/yellowtail	Asia	Yes Yes
Channel catfish virus disease/CCV	Channel catfish	USA	No
Spring viremia of carp: /SVCV	Mostly carp species	Europe	No*
Grass carp hemorrhage disease/GCHDV	Grass carp	China	Yes‡

*Previously available inactivated virus vaccine but no longer commercially available; ‡Previously available but may not be in use today.

CCV: Channel catfish virus; GCHDV: Grass carp hemorrhage disease virus; IHNV: Infectious hematopoietic necrosis; IPNV: Infectious pancreatic necrosis virus; ISAV: Infectious salmon anemia; PDV: Pancreas disease virus; RSIV: Red sea bream iridovirus: SJNNV: Striped jack nervous necrosis virus: SVCV: Spring viremia of carp virus; VHSV: Viral hemorrhagic septicemia virus.

Table 3. Examples of some major parasitic diseases in fish in relation to vaccine availability.

Pathogen(s)/disease	Major fish species affected	Primary region(s)/country
Amoebae		
Paramoeba spp. (Amoebic gill disease)	Salmonids	Europe, Asia, America, Australia
Flagellates		
Cryptobia salmositica	Salmonids	North America
Ichthyobodo spp.	Various fish	Globally
Ciliates		
Ichthyophthirius multifilis (White spot disease)	FW fish	Globally
Cryptocaryon irritans	SW fish	Globally
Trichodina spp.	Various fish	Globally
Microsporidia		
Tetramicra brevifilum	Turbot	Europe
Pleistophora anguillarum	Japanese eel	Japan
Nucleospora salmonis	Salmonids	North America
Myxosporeans		
Myxobolus cerebralis (Whirling disease)	Salmonids, FW	Europe, North America
Tetracapsula bryosalmonae (proliferative kidney disease; PKD)	Salmonids	Europe
Kudoa thyrsites	Salmonids	North America (West coast)
Monogeneans		
Gyrodactylus spp.	Various fish	Globally
Dactylogyrus spp.	Various fish	Globally

Characteristics of vaccines

- Safety
- Immunogenicity
- Stimulation of protective immunity
- Efficacy of Vaccines
- Specificity

- **INACTIVATED/KILLED VACCINE**
- Inactivated vaccine is prepared by physical or chemical treatment to the pathogen so that the organisms become inactive (loses replication capability) but maintains its immunogenicity.
- The procedure should not disturb the immunogenic structures or epitopes, but should remove the replication or virulence of the organisms. This vaccine is usually prepared with a virulent strain and the vaccine is more immunogenic.
- In general these kinds of vaccine are used when attenuated vaccines are not available or for an outbreak

- where characterization of the organism is not determined and pathogenicity have not been assessed.
- Examples of chemical inactivating agents are formaldehyde, glutaraldehyde, beta propiolactone etc., they change the structural conformation or cross link the structures and ultimately inactivate the organisms.
- The physical inactivating agents are gamma irradiation, U-V irradiation etc. which are going to change the structural conformation or cross – linking structures.
- In general, inactivated vaccine requires an adjuvant to increase the potency of the vaccine.

- Advantages
  - No possibility of reversion
  - No shedding and contamination of environment
  - Quite stable, thus less need for cold chain
  - More immunogenic
  - Whole organism has both T and B epitopes.

- Disadvantages
  - Cannot replicate so antigen is limited
  - Require, multiple doses, adjuvants and boosters vaccination
  - If not properly inactivated, it may cause disease outbreaks
  - Increased risk of allergic reactions due to large amounts of antigen involved
  - Costly
  - May be ineffective against intracellular organisms.

- **LIVE ATTENUATED VACCINES**
- The virulence of the pathogen is reduced and immunogenicity is maintained by adapting the pathogen in an unfavourable condition and the organism still replicates.
- Attenuation is achieved by growing the pathogens in an unnatural host, by passaging in non homologous host (host/cell culture) for repeated period of time (i.e. 70-80 times) or in different physiological conditions or in different environment.

- Attenuation may also be done by adapting the virus to grow in a temperature lower than the normal called cold adapted virus and the process is called cold adaption.
- Thermo stable vaccine strain grows at elevated temperature.
- Temperature sensitive mutants cannot grow at slightly elevated temperature.
- The process of reducing the virulence and retaining the immunogenicity is called as attenuation so that the pathogen changes its habit of growing.

- Advantages
  - Replication provides large quantities of immunogen
  - There is no need for adjuvant
  - Single dose often produce long lasting immunity
  - Whole organism has both T and B epitopes
  - The vaccine is cost effective and often does not require booster vaccination
  - Can be effective against intracellular pathogens

- Disadvantages
  - Chance of reversion to virulence
  - There may be shedding of virus
  - Can induce transient immunosupression
  - Cold chain required for transport
  - Possible contamination with other animal viruses
  - There may be side effects due to unwanted parts of the vaccines.

- **Adjuvants** provide depots effect to an antigen at the site of administration which allows persistence and slow release of antigen over an extended period of time resulting in higher and prolonged immune response.
- Adjuvants increase immunogenicity of weak antigens.
- It helps in stimulation of cell-mediated immune response.
- Addition of adjuvant reduces the cost and dose of an antigen.
- *Examples*, mineral oils (Aluminium hydroxide, Liquid paraffin etc.) Vegetable oils (Ground Nut oil, Montanide etc.), Mycobacterial products (Freund's adjuvant) etc.
- Some other delivery systems are ISCOM (Immunostimulating complex), Nanoparticles etc.

- **TOXOID**
- Both gram negative and gram-positive bacteria produce exotoxins. Exotoxins can be inactivated by formaldehyde, iodine, other chemical or heat treatment & form **toxoid.**
- Toxoid is immunogenic without toxic effects. Toxoid vaccines have been used for tetanus, anthrax etc.
- Some veterinary vaccines combine both toxoid and killed bacteria by formalinizing whole culture and this is called anaculture. These types of vaccines are available for clostridial diseases.

- Trypsinization of anaculture makes it more immunogenic.
- *Advantage:* The exotoxin is immunogenic and whole organism can be avoided.
- *Disadvantage:* Only effective if diseases caused solely by bacterial exotoxins.

- **BACTERINS**
- Bacterins are the vaccines containing killed bacteria. This is usually done with formal dehyde and adjuvant like aluminiam hydroxide or alum is added to increase its immunogenicity.
- Autogenous vaccines are prepared using the organism from the infected animal itself or from other infected animals in the same farm after inactivation with formal dehyde and found successful to control diseases. For example fowl cholera vaccine.
- *Advantages:* Easy to prepare ,No reversion to virulence
- *Disadvantages:* Immunity is short lasting (usually less than six months)

- **SUBUNIT VACCINES/CONJUGATE VACCINES**
- It is possible to identify the peptide sites encompassing the major antigenic sites of viral antigens, from which highly purified subunit vaccines can be produced. But increasing purification may lead to loss of immunogenicity, and this may necessitate coupling to an immunogenic carrier protein or adjuvant.
- *Example* of a purified subunit vaccine is HA vaccines for influenza A and B. Bacterial capsular polysaccharides are immunogenic but incapable of evoking T cell responses.
- Vaccines efficacy can be greatly increased by conjugating the capsular polysaccharide to a protein carrier capable of supply of T cell epitopes called a conjugate vaccine.

- Advantages
  - Avoids use of whole organism
  - Side effects due to undesired part of the organism is reduced
  - Supplies multiple epitopes.
- Disadvantages
  - Possible alteration of pathogen protein conformation during purification may decreases immunogenicity
  - Can be laboured intensive and costly to purify immunogens
  - May require cold chain
  - Sometimes too large to fit into the vaccine delivery systems.

PEPTIDE VACCINE

- Once the immunogenic sites of an organism are identified, immunogenic peptides can be synthesized or can be purified from natural sources.
- Several methods have been used to prepare it.
- Synthetic peptide vaccines would have many advantages. Their antigens are precisely defined and free from unnecessary components which may be associated with side effects.
- They are stable and relatively cheap to manufacture.
- *Example*, foot and mouth disease peptide vaccine where protection was achieved by immunizing animals with a linear sequence of 20 amino acids (141 to 160) of VP1.
- Synthetic peptides do not readily stimulate T cells and require coupling to a protein carrier which is recognized by T-cells.

- Advantages
  - Avoids use of whole organism
  - Side effects due to undesired part of the organism is reduced
  - Small enough to fit into most the antigen delivery vehicles
  - Quite stable
- Disadvantages
  - May be perceived as haptens if not conjugated to carriers
  - Rapidly dissipated in tissues, thus requires highly effective adjuvants or effective delivery vehicles.
  - May be costly or difficult to identify and purify.

- The immune dominant part of a pathogen is cloned into a vector and pathogen DNA is transcribed and translated within the cells of vaccinated animals.
- Virus proteins have been expressed in bacteria, yeast, mammalian cells, and viruses.
- *E. coli* cells were first to be used for this purpose but the expressed proteins were not glycosylated, which was a major drawback since many of the immunogenic proteins of viruses such as the envelope glycoproteins, were glycosylated.
- An alternative application of recombinant DNA technology is the production of hybrid virus vaccines.

- Recombinant technology made some useful safe virus vectors for the expression of protective antigens from potentially harmful infectious agents.
- Compared to the subunit vaccines the vectored vaccines produces good immune responses against various pathogens.
- Poxviruses, adenoviruses, herpes viruses are commonly used as vectors for vaccines.Examples of vector based recombinant vaccine, ND virus in fowl pox virus, Rabies virus in vaccinia virus etc..
- Recombinant hepatitis B vaccine is a licensed vaccine.

- Advantages
  - Use of pathogens can be avoided
  - Unwanted reaction is reduced
  - High immune response.
  - Hybrid virus vaccines are stable and stimulate both cellular and humoral immunity.
  - They are relatively cheap and simple to produce.
- Disadvantages
  - Replication of vector may induce side effects
  - Primary immune responses mounted against vector proteins may generate anti – vector antibodies that blocks booster immunization.

- **DIVA/MARKER VACCINES**
- Vaccination employing conventional vaccines interferes with the serological detection of infection with the pathogens and thus in the assessment of prevalence and incidence of diseases.

- This necessitates the development of DIVA vaccines that are capable of distinguishing between antibody responses resulting due to vaccination and infection (DIVA- Differentiating infected from vaccinated individuals) or marker vaccines.

- **DIVA/MARKER VACCINES**
- A marker vaccine (live or inactivated) is either based on deletion mutant or isolating antigenic proteins that allows the distinction between vaccinated and infected animals on the basis of identifiable differences in antibody responses.
- A marker vaccine is used in conjuction with a test that detects antibodies against protein that is lacking in the vaccine strain.
- DIVA vaccine was useful to control avian influenza in Italy.