GENETIC VACCINES - APPLICATION IN VETERINARY MEDICINE

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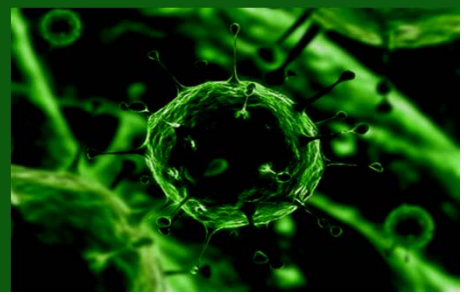
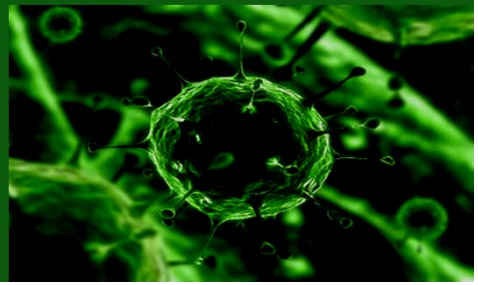


Based on the biological properties and the safety features the vaccines are divided into 3 groups:

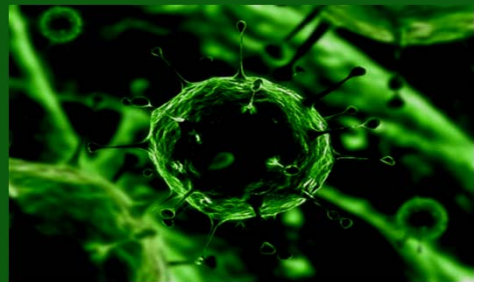
I. group that includes non-viable or killed products that pose a minor risk to the environment.

II. group of vaccines containing living microorganisms, modified by the addition or deletion of one or more genes.

III. group using live vectors for transferring recombinant foreign genes encoding the immunizing antigens.



- Vaccines can prevent the effects of infection by many pathogens. They are antigenic material that stimulate adaptive immunity to a diseases. Vaccine's are generally considered to be the most effective method of preventing infectious diseases.
- The material administered can either be killed or inactivated forms of bacterial or virus antigens.
- The material administered can be attenuated forms of bacteria or viruses.
- The material administered can be purified materials such as proteins.



INACTIVATED VACCINES

An inactivated vaccine consists of virus particles which are grown in culture and then killed using chemical or physical methods as treatment with formaldehyde or heat.

The virus particles are destroyed and cannot replicate, but the virus proteins are intact and can be recognized and remembered by the immune system and evoke a immune response.

When manufactured correctly, the vaccine is not infectious, but improper inactivation can result in intact and infectious particles and danger from vaccine infection.

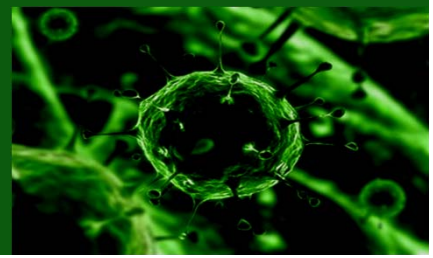
The correctly produced vaccine does not reproduce infection in animals, but they are less immunogenic and booster are required periodically to reinforce the immune response.

LIVE ATTENUATED VACCINES

In live attenuated vaccine, live virus particles with very low virulence are administered in animals. They are prepared from mildly virulent field isolates of bacteria or viruses or by their passage through laboratory animals, cell cultures or hen embryos.

By the fact that they are live attenuated organisms they reproduce slowly. Since they do reproduce and continue to present antigen beyond the initial vaccination, less often are required boosters.

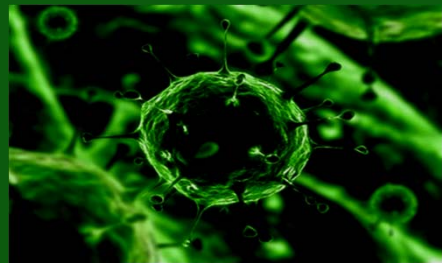
•If the immunogen in the vaccine is definitely attenuated the risk of reversion to virulence is small.



HIGHLY PURIFIED VACCINES

Preventive vaccination against foot and mouth disease in EC has been prohibited since 1991. In order to overcome the risk from FMD infection have been established vaccine banks, containing highly purified FMD virus antigens. In case of emergency outbreaks there exist possibility to use the purified virus antigens as marker vaccines. FMD virus contain structural and non structural proteins (NSP). During vaccine preparation the NSP are removed by antigen purification.

Differentiation of vaccinated from infected animals (DIVA strategy) are performed by using 3 ABC ELISA diagnostic kit. The absence of seroconversion to NSP can be found in vaccinated animals . During the virus replication in cell culture and infected animals NSP are produced. The presence of seroconversion to NSP means infected animals.



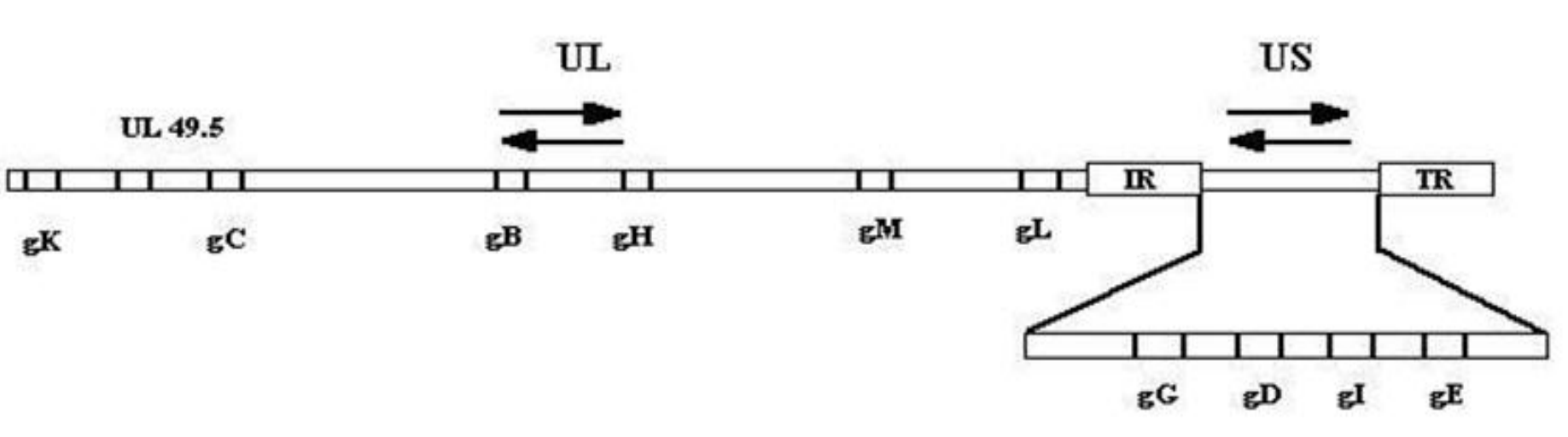
MARKER VACCINES FOR HERPES VIRUSES

By using molecular biological methods are produced marker vaccines. Marker vaccines are obtained by deletion or insertion of certain genes, or genes that encode specific immunizing antigens from the microorganism. They are associated with companion diagnostic methods.

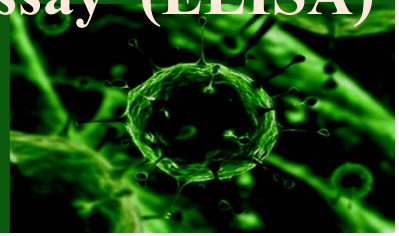
Such vaccines are used for eradication of pseudorabies and infectious bovine rhinotracheitis. First marker vaccine against pseudorabies virus has spontaneous deletion in gE gene. By using molecular methods deletion in gI/gE, gD or Tk genes can be produced. Marker vaccines can be inactivated or attenuated. The name of this strategy is DIVA strategy. With acompanied diagnostic test differentiation of vaccinated from infected animals can be accomplished.

MARKER VACCINES FOR HERPES VIRUSES

The glycoprotein E is not essential for viral replication, but it has a major role in intercellular spread, particularly along the nerves.

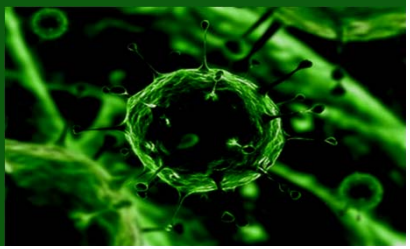


For determination of immune status specific diagnostic tests based on gE deletion have been developed using both gE-blocking enzyme-linked immunosorbent assay (ELISA) techniques and PCR amplification.



VECTOR VACCINES

Herpes viruses, adenoviruses and poxviruses containing large genome are used as delivery systems (vectors) for insertion of foreign genes. Those viruses can be used simply as a vector or simultaneously as a vector and vaccine. Example for this is the recombinant capripox virus expressing virus antigens from PPRV. The advantages of virus vectors are high efficiency of gene transduction, specific delivery of genes to target cells and induction of robust immune responses. The plasmids containing recombinant transgene are transfected into virus vector. The recombinant vectors are cultivated in cell culture and the final products are cleared by centrifugation. An example for viral vector vaccine is the oral vaccine against rabies virus in the form of a bait containing a recombinant vaccinia virus vector expressing the protective glycoprotein G of rabies virus. Viral vector vaccines differ from subunit vaccines. Subunit vaccines prevent infectious diseases by eliciting a humoral response. Recombinant viral vector vaccines induce a robust cytotoxic T lymphocyte (CTL) response, leading to the elimination of virus-infected cells.

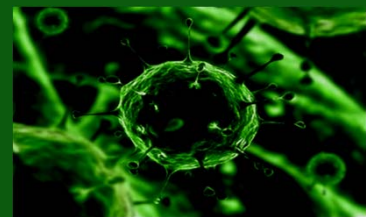


SUBUNIT VACCINES

By genetic engineering techniques are produced vaccines which use only the parts of an organism which stimulate a strong immune response. For creation of subunit vaccine the gene or genes coding appropriate subunits from the genome of the infectious agent are used. The genetic material is placed into bacterial, viral or yeast cells which then produce large quantities of subunit molecules by transcribing and translating the inserted foreign DNA. These foreign molecules can be isolated, purified, and used as a vaccine. An example of subunit vaccine are vaccines against classical swine fever and Hepatitis B virus. The hepatitis B genes that code for the antigens were inserted into common baker's yeast. The yeast grew and expressed the genes and produced the antigen protein.

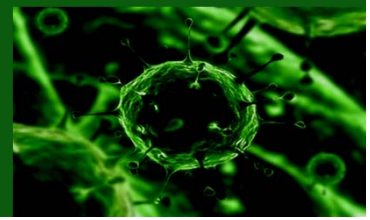
SUBUNIT VACCINES

Other method of subunit vaccines production involves isolation of a specific proteins from a viruses or bacteria and administering this by itself. A subunit vaccine presents an antigen to the immune system without introducing viral particles, whole or otherwise. A weakness of this technique is that isolated proteins may have a different three dimensional structure than the protein in its normal context, and will induce antibodies that may not recognize the infectious organism. In addition, this type of subunit vaccines often elicit weaker antibody responses than the other classes of vaccines.



SUBUNIT VACCINES

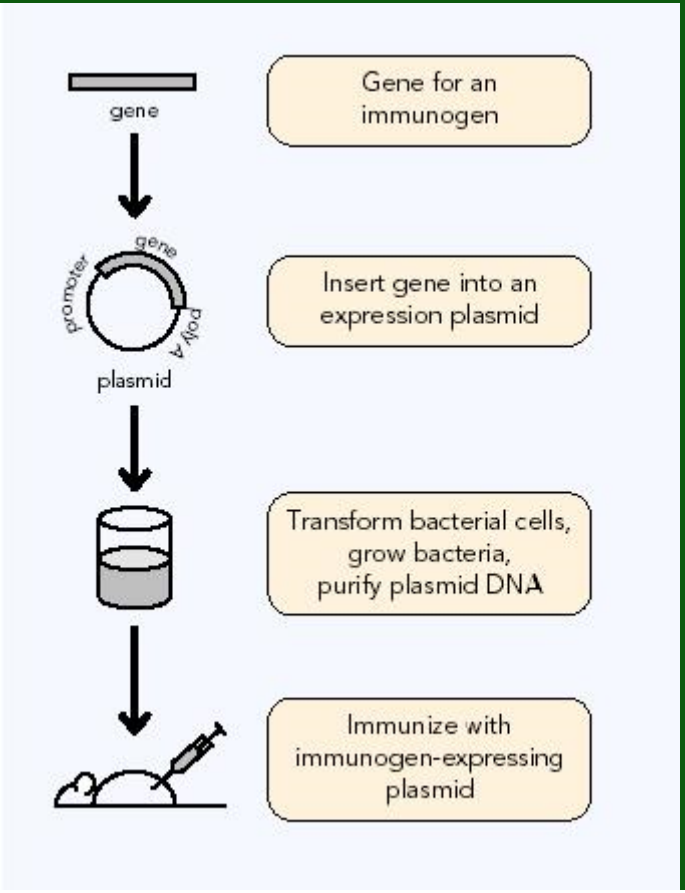
The genome of CSFV consists of a single-stranded, positive-sense RNA with a single open reading frame (ORF). It code a polyprotein which is cleaved into 11 mature viral proteins. Four proteins including nucleocapsid protein C and three envelope glycoproteins E^{rns}, E1, and E2 are structural proteins. E2 is the most immunogenic protein in the envelope of CSF virus and plays an important role in virus neutralization. C DNA sequences coding E2 proteins are inserted in baculovirus system, vacinia or pseudorabies viruses. The baculovirus vaccine allows distinction between vaccinated and infected swine after using companion diagnostic tests. They are designed to detect antibodies directed against other immunogens not participating in the vaccine for example conservative NS 2 protein.

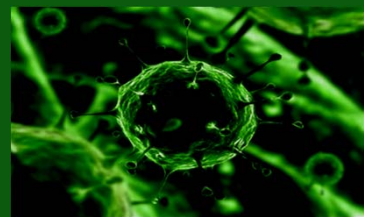


DNA VACCINES

Direct introduction into host cells of a bacterial plasmid DNA expressing an antigenic protein under the control of eukaryotic cell promoter is DNA vaccination.

The principle scheme for production of DNA vaccines is:

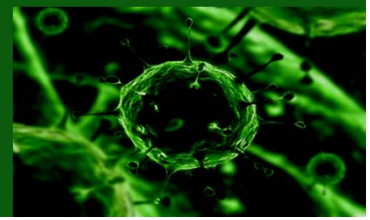




DNA VACCINES

After injection in muscle by Gen Gun gold particles containing plasmid DNA coated on it, DNA of interest is integrated into DNA of target cells. After foreign DNA transcription is produced mRNA which is transported from nucleus, translated and expressed foreign antigen, which is processed by pathway. The antigenic peptides are displayed on cell surface, presented on the host cell MHC and evoked cell mediated and humoral immune response.

DNA vaccine has some benefit of live vaccine and other advantages as cost, easy production and absence of risk associated with live vaccines. The disadvantages of DNA vaccine are long time for plasmid persistence in the organism and the potential for integration of plasmid in chromosome. By simultaneous inoculation of immunostimulators the immune response can be increased. Currently different type of DNA vaccine are developed – against anthrax, tuberculosis, influenca, denga hepatitis B, West Nile fever virus.



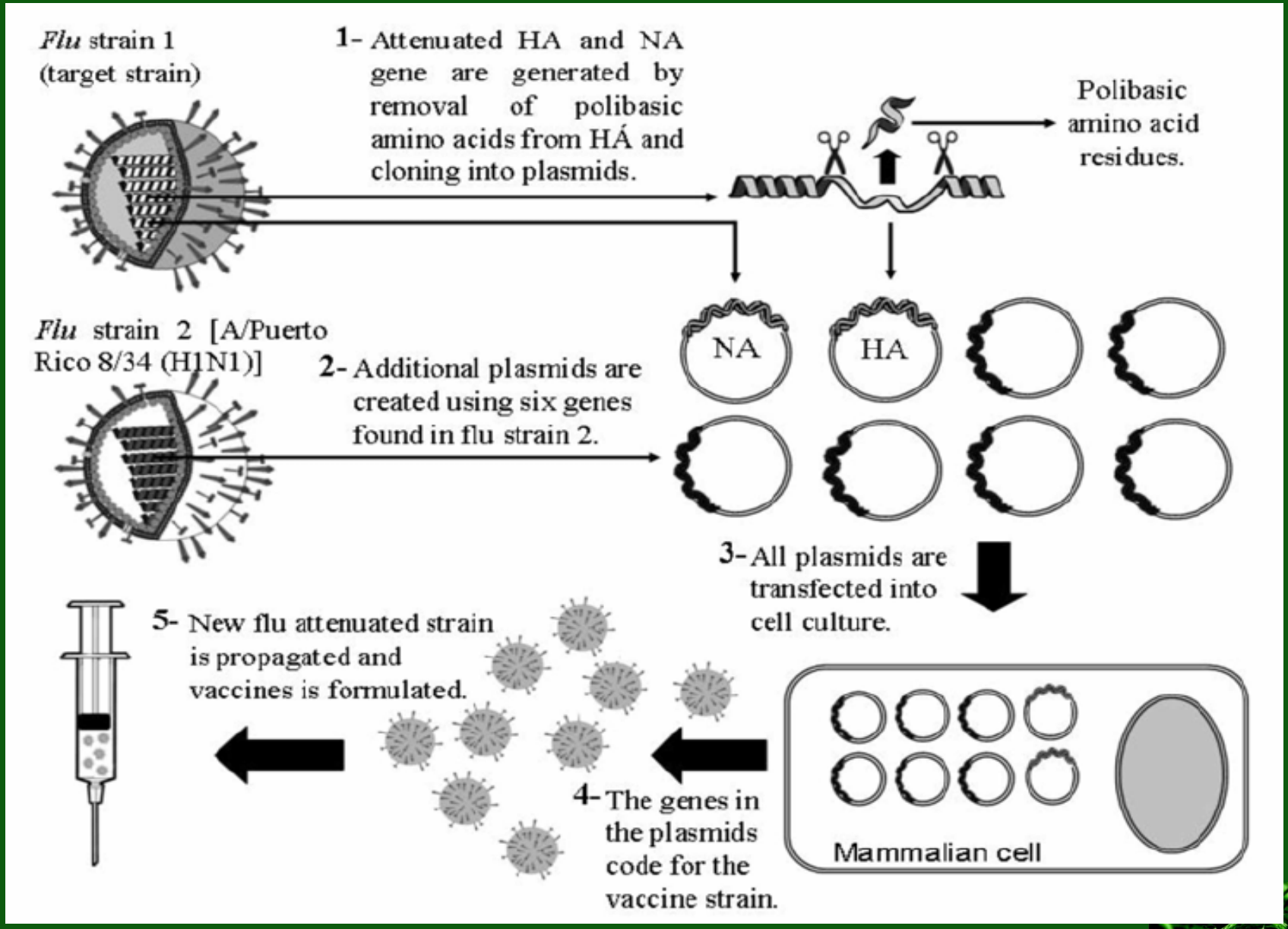
PLANT (EDIBLE) VACCINES

One way of generating edible vaccines relies on the bacterium Agrobacterium tumefaciens to deliver into plant cells the genetic blueprints for viral or bacterial "antigens"—proteins that elicit a targeted immune response in the recipient. The production of plant vaccines in potatoes has the following succession.

- ***1 Cut leaf.***
- ***2 Expose leaf to bacteria carrying an antigen gene and an antibiotic- resistance gene. Allow bacteria to deliver the genes into leaf cells.***
- ***3 Expose leaf to an antibiotic to kill cells that lack the new genes. Wait for surviving (gene-altered) cells to multiply and form a clump (callus).***
- ***4 Allow callus to sprout shoots and roots.***
- ***5 Put in soil. Within three months, the plantlets will grow into plants bearing antigen-laden vaccine potatoes.***

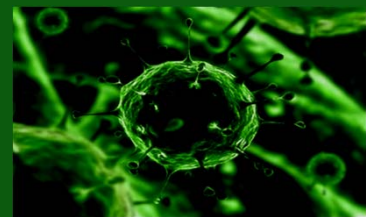
The plant vaccines would activate both mucosal and systemic immunity. High priority is development of vaccines against viruses and bacteria causing diarrhea as Norwalk virus, rotavirus, Vibrio cholerae and enterotoxigenic Escherichia coli (a toxin-producing source of "traveler's diarrhea").

VACCINES AGAINST INFLUENCA VIRUS



LEGISLATION CONNECTED WITH VACCINES

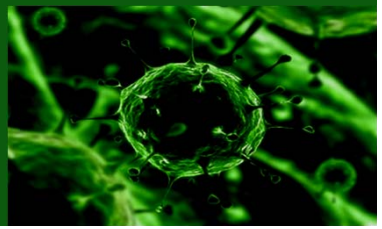
European regulatory requirements for medicinal products are governed by Regulation (EC) No 726/2004 (centrally authorized products), Directive 2001/82/EC (nationally authorized products for veterinary use), and Directive 2001/83/EC (nationally authorized products for human use). The technical requirements for veterinary vaccines are laid down in Annex 1, Title II, to Directive 2001/82/EC as amended by Directive 2009/9/EC. Annex 1 also includes a requirement for vaccines to comply with the requirements of the European Pharmacopoeia (Ph. Eur.). The Ph. Eur. is published by the European Directorate for the Quality of Medicines and Healthcare (EDQM) of the Council of Europe. It is composed of monographs and other texts that define quality standards applicable to medicinal products, including vaccines, in Europe.



ENVIRONMENTAL RISK ASSESSMENT

To assess the environmental risk of using biotechnology-derived vaccines two approaches are available: a general conventional approach ; and an approach which is specific to biotechnological products.

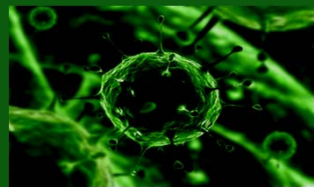
- General conventional approach:
- Should the replication of a vaccine strain be limited in the vaccinated organism? What are the accepted limits?
- Is it acceptable for vaccine strains to be potentially diffused from vaccinated animals to non-vaccinated animals ? What are the acceptable limits ?
- Which administration route is to be used ? What are the risks from virulence reversion?
- What is recombination risks with other vaccine strains and wild strains ?



ENVIRONMENTAL RISK ASSESSMENT

Approach specific to biotechnological products:

The risk depends of the nature of new generation vaccines for example: The risk associated with recombinant vaccines expressed in bacteria or yeast are connected with the processes of purification, as antigenic fractions can be 'polluted' by vectors which were not totally eliminated in the finished product. The risk in using antigens expressed in a replicative vector is directly associated with the vector used, and is therefore dependent on the biological properties of this vector in the target host and potential non-target hosts. In gene deleted vaccines the virulence should be considerably decreased or even totally suppressed. When assessing the risk associated with using a vaccine product, this risk should always be balanced against the benefit to be derived from use of the product.



THANK'YOU FOR ATTENTION