(Review Article)

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Analysis of Genetic Engineering Product "Vaccines"

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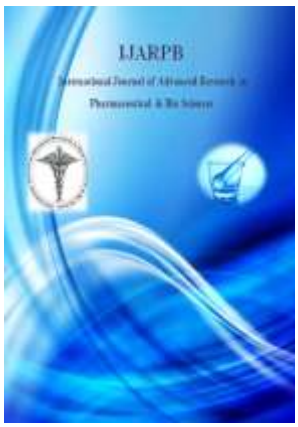

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ABSTRACT

Vaccines are preparations of antigenic substances that are administered for the purpose of inducing in the recipient a specific and active immunity against the infective agent or toxin produced by it. Vaccines may contain living micro-organisms suitably treated to attenuate their virulence but retain their antigenic potency or they may consist of pathogenic organisms which have been killed or inactivated. Some vaccines consist of antigenic fractions or substances produced by the same pathogenic organisms but rendered harmless whilst retaining their antigenic efficiency. Vaccines may be prepared from one species only or from a mixture of two or more species genetic engineering play an important role for production, development, and analysis of vaccines. The quality control of vaccines is intended to provide assurances of both the probable efficacy and the safety of every batch of every product. It is executed in three ways in process control Final product control; and A requirement that for each product the starting materials, intermediates, final product and processing methods are consistent. Different official and non official methods used for analysis of vaccines

KEY WORDS: Vaccine, Quality control, Genetic engineering, Immunization

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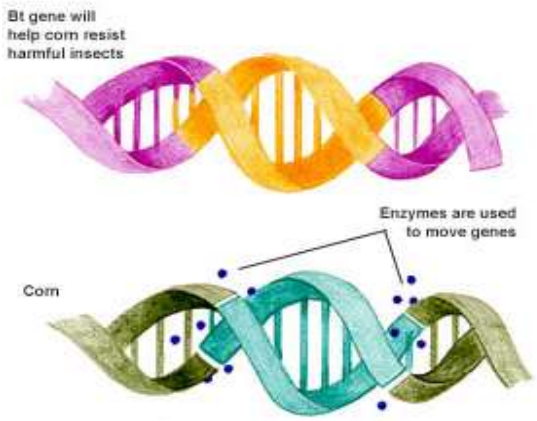
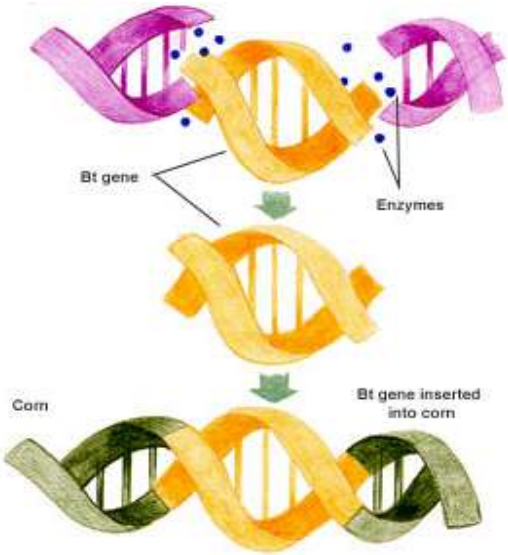
INTRODUCTION- GENETIC ENGINEERING

Genetic engineering is a laboratory technique used by scientists to change the DNA of living organisms. Genetic engineering, also known as recombinant DNA technology, means altering the genes in a living organism to produce a Genetically Modified Organism (GMO) with a new genotype.

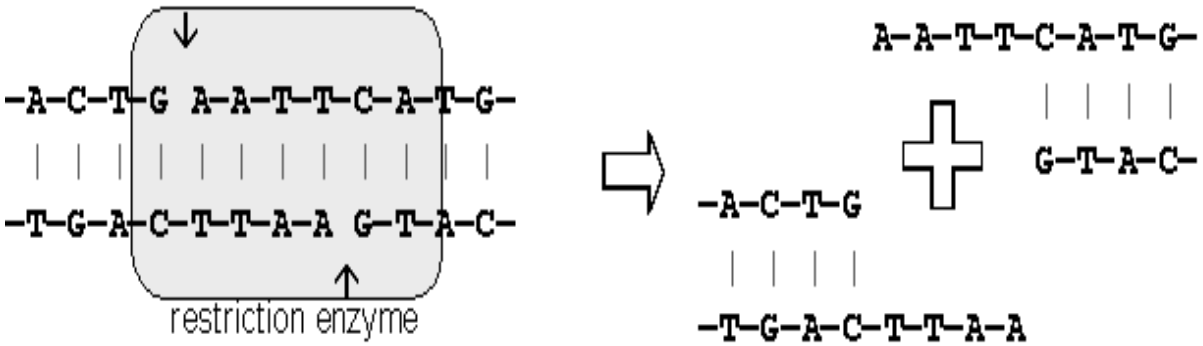
Various kinds of genetic modification are possible like inserting a foreign gene from one species into another, forming a transgenic organism, altering an existing gene so that its product is changed or changing gene expression so that it is translated more often or not at all[1].





Process: - Basic steps in genetic engineering

Step 1: Isolating the gene



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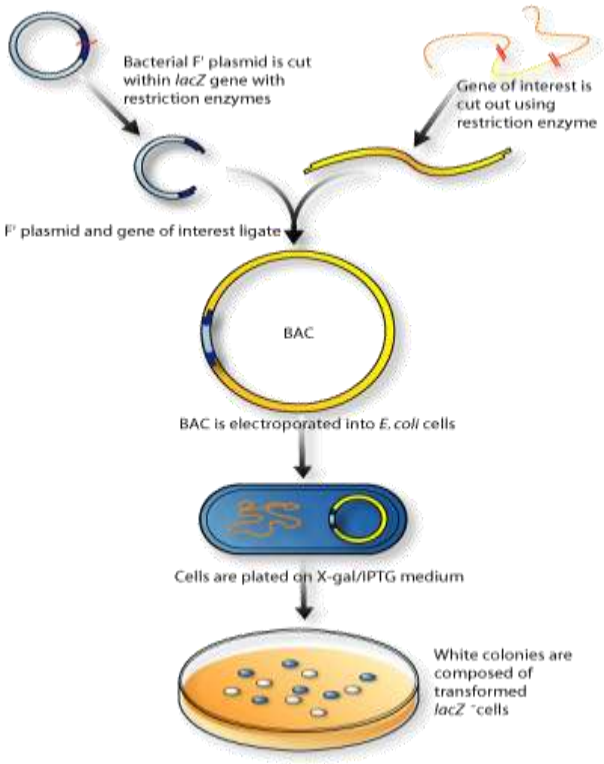
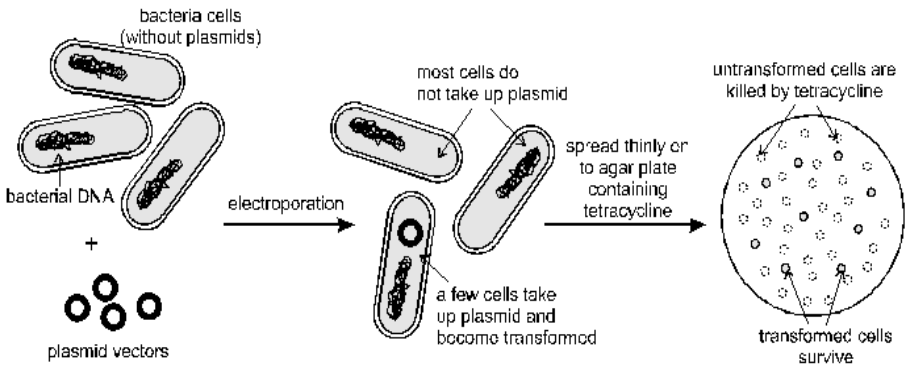
Step 2: Inserting gene into vector

Vector – molecule of DNA which is used to carry a foreign gene into a host cell



Step 3: inserting vector into host



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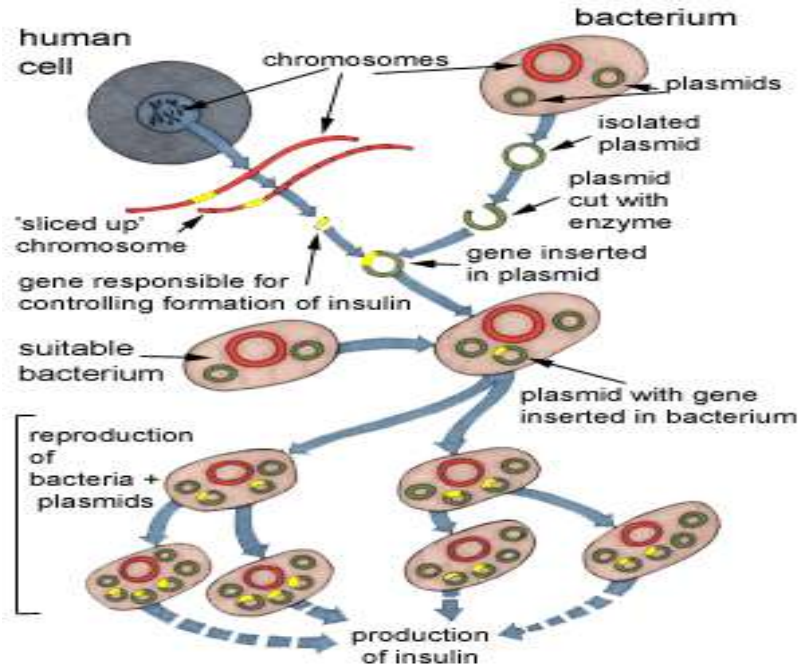
Step 4: Multiplication of the host cells by cloning

Large scale fermenters by cloning and all cells are genetically identical because of asexual reproduction



GENETIC ENGINEERING PRODUCT VACCINES

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 or its toxins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "recognize" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters[2].

Different Kinds of Vaccines

Killed Whole Organisms

In this relatively crude approach, the vaccine is made from the entire organism, killed to make it harmless. The typhoid vaccine is an example.

Attenuated Organisms

Here, the organism has been cultured so as to reduce its pathogenicity, but still retain some of the antigens of the virulent form. The Bacillus Calmette-Guérin (BCG) is a weakened version of the bacterium that causes tuberculosis in cows. BCG is used as a vaccine against tuberculosis in many European countries but is rarely used in the U. S[3].

Toxoids

In some diseases, **diphtheria** and **tetanus** are notorious examples; it is not the growth of the bacterium that is dangerous, but the protein toxin that is liberated by it. Treating the toxin with, for example, formaldehyde, denatures the protein so that it is no longer dangerous, but retains some epitopes on the molecule that will elicit protective antibodies.

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Surface Molecules

Antibodies are most likely to be protective if they bind to the surface of the invading pathogen triggering its destruction. Several vaccines employ purified surface molecules:

Influenza vaccine contains purified hemagglutinins from the viruses currently in circulation around the world.

The gene encoding a protein expressed on the surface of the hepatitis B virus, called hepatitis B surface antigen or **HBsAg**, can now be expressed in E.coli cells and provides the material for an effective vaccine. Hepatitis B infection is strongly associated with the development of liver cancer. Here then is a vaccine against a cancer.

The genes encoding the capsid proteins of 4 strains of human papilloma virus (**HPV**) can be expressed in yeast and the resulting recombinant proteins are incorporated in a vaccine (Gardasil®). Because infection with some of these strains of HPV can lead to cervical cancer, here is another vaccine against cancer.

Some 80 different strains of Streptococcus pneumoniae cause pneumonia in humans. They differ in the chemistry of the polysaccharide capsule that surrounds them (and makes it difficult for phagocytes to engulf them by endocytosis). The current vaccine consists of tiny amounts of the purified capsular polysaccharides of the 23 most common and/or dangerous strains.

Inactivated Virus

Like killed bacterial vaccines, these vaccines contain whole virus particles that have been treated (again, often with formaldehyde) so that they cannot infect the host's cells but still retain some unaltered epitopes. The Salk vaccine for polio (IPV) is an example.

Attenuated Virus

In these vaccines, the virus can still infect but has been so weakened that it is no longer dangerous. The measles, mumps, and rubella ("German measles") vaccines are examples. The Sabin oral polio vaccine (OPV) is another example. It has advantages over the Salk vaccine in that it is given by mouth rather than by injection; the viruses it contains can spread to the other members of the vaccinee's family thus immunizing them as well[4, 5].

QUALITY CONTROL ANALYSIS- GENERAL CONSIDERATION

Quality control

The quality control of vaccines is intended to provide assurances of both the probable efficacy and the safety of every batch of every product. It is executed in three ways:-

In process control
Final product control; and
A requirement that for each product the starting materials, intermediates, final product and processing methods are consistent.

In process quality control

In process quality control is the control exercised over starting material and intermediates. Its importance stems from the opportunities that it provides for the examination of a product at the stages in the most meaningful information. The WHO recommendations and national authorities stipulate many in-process controls but manufacturer often perform test in excess of those stipulated, especially sterility tests as, by doing, they obtain assurance that production is

proceeding normally and that he final product is likely to be satisfactory[6]

Final product control

Quality control analysis of final product is carried out using different official and non official methods but mostly methods follow same principle discussed under common methods. IP/BP methods (official methods) for different types of vaccines used for analytical purpose to determine the Efficacy, Safety, Quality of vaccine products. Different methods used to control quality of final product as

ASSAYS METHOD

For vaccines containing killed microorganisms or their products-

These are generally tested for potency in assays in which the amount of the vaccine that is required to protect animal from a defined challenge dose of the appropriate pathogen, or its product, is compared with the amount of a standard vaccine that is required to provide the same protection.

The usually format of the test is the 3+3 dose quantal assay that is used to estimate the potency of whole cell pertusis vaccine the logarithmic serial dose of the test vaccine and three the logarithmic serial dose of the standard vaccine are made and each is used to inoculate a group of 16 mice[8].

In the case of both test and standard vaccine, the middle dose chosen on the basis of experience, so that it is sufficient to induce a protective response in about 50% of the mice animal to which it is given. Each lower dose may then be expected to protect <50% of the mice to which it is given and each higher dose to protect >50% of the animals. Fourteen day later all of the mice are inoculated (challenged) with virulent Bordetella pertussis and, after a further 14 day, the number of mice surviving in each of the six groups is counted.

The number of survivors in each group is used to calculate the potency of the test vaccine relative to the potency of the standard vaccine by the statistical method of probit analysis. The potency of the test vaccine may be expressed as a percentage of the potency of the standard vaccine. However, as the standard vaccine will have an assigned potency in international units.

For vaccines containing live microorganisms-

These are generally tested for potency by determining their content of viable particles. In the case of the most widely used live bacterial vaccine, BCG vaccine, dilutions of vaccine are prepared in a medium which inhibits clumping of cells, and fixed volumes are dropped on to solid media capable of supporting mycobacterial growth. After a fortnight the colonies generated by the drops are counted and the live count of the undiluted vaccine is calculated. The potency of live viral vaccines is estimated in much the same way except that a substrate of living cells is used. Dilution of vaccine are inoculated on to tissue culture monolayer's in Petri dishes or in plastic trays, and the infective particle count of the vaccine is calculated from the infectivity of the dilutions are indicated by plaque formation, cytopathic effect, haemadsorption or other effect and the dilution factor involved.

Safety tests

Because many vaccines are derived from basic materials of intense pathogencity-the lethal dose of tetanus toxin for a mouse is estimated to be $3*10^{-5}$ µg–safety testing is of paramount importance. Effective testing provides a guarantee of the safety of each batch of every

product and most vaccines in the final container must pass one or more safety tests as prescribed in a Pharmacopoeial monograph.

Bacterial vaccines are regulated by relatively simple safety tests. Those vaccines composed of killed bacteria or bacterial products must be shown to be completely free from the living microorganisms used in the production process[9].

Viral vaccines can present problems of safety testing far more complex than those experienced with most bacterial vaccines. With killed viral vaccines the potential hazards are those due to incomplete virus inactivation and the consequent presence of residual live virus in the preparation .The tests used to detect such live virus consist of the inoculation of susceptible tissue cultures and of susceptible animals. The cultures are examined for cytopathic effects, and the animals for symptoms of disease and histological evidence of infection at autopsy. This test is of particular importance in inactivated poliomyelitis vaccines, the vaccine being injected intraspinally into monkeys. At autopsy, sections of brain and spinal cord are examined microscopically for the histological lesions indicative of proliferating poliovirus[10].

Tests of general application11

In addition to the tests designed to estimate the potency and to exclude the hazards peculiar to each vaccine there are a number of tests of more general application .These relatively simple tests are as follows.

Sterility

In general, vaccines are required to be sterile. The exceptions to this requirement are smallpox vaccine made from the dermis of animals and bacterial vaccines such as BCG, Ty21A and tularaemia vaccine, which consist of living but attenuated strains. These have a bioburden limit which defines the number of permissible microorganisms but excludes pathogens. WHO recommendations and pharmacopoeial monographs stipulate, for vaccine batches of different size, the numbers of containers that must be tested and found to be sterile. The preferred method of sterility testing is membrane filtration, as this technique permits the testing of large volumes without dilution of the test media. The test system must be capable of detecting aerobic and anaerobic bacteria and fungi.

Freedom from abnormal or general toxicity-

The purpose of this simple test is to exclude the presence in a final container of a highly toxic contaminant. Five mice of 17-22g and two guinea-pigs of 250-350g are inoculated with one human dose or 1.0ml, whichever is less, of the test preparation. All must survive for 7days without signs of illness. Current pharmacopoeial monographs usually do not require this test if another in vivo test has been performed on the product[11, 12].

Pyrogenicity or endotoxin content-

The pyrogenicity of a specified dose of product when administered to rabbits can be assayed by a standard pharmacopoeial method but the trend is to replace this with an in vitro assay for endotoxin. The capacity of the product to induce gelation of Limulus polyphemus amoebocyte lysate is determined against a reference endotoxin preparation and the result is expressed as IU of endotoxin[13].

Presence of aluminium and calcium-

The quantity of aluminium in vaccines containing aluminium hydroxide or aluminium phosphate as an adjuvant is limited to 1.25mg per dose and it is usually estimated complexiметrically. The

quantity of calcium is limited to 1.3mg per dose and is usually estimated by atomic absorption spectrometry[14, 15].

Free formaldehyde

Inactivation of bacterial toxins with formaldehyde may lead to the presence of small amounts of free formaldehyde in the final product. The concentration, as estimated by colour development with acetyl acetone, must not exceed 0.02%.

Phenol concentration-

When phenol is used to preserve a vaccine its concentration must not exceed 0.25% w/v or, in the case of some vaccines, 0.5% w/v. phenol is usually estimated by the color reaction with amino-phenazone and hexaeyanoferrate[16, 17].

CONCLUSION

A growing number of vaccines today are based on well-defined materials, including vaccines based on purified protein antigens of natural origin or produced by rDNA technology, polysaccharides, semi-synthetic poly- or oligosaccharide-protein conjugates and novel nucleic acid constructs. These products can raise challenges for characterisation. Through the application of a comprehensive suite of methods currently in use for evaluating carbohydrate, lipopolysaccharide, lipid, protein, glycoprotein, and lipoprotein components of vaccines. The analysis of vaccines to estimated the benefits, risks and costs of routine childhood immunization against particular disease.

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