Production Process and Quality Control of Virus Vaccine

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Abstract: According to the current hot demand of the society, the importance of human vaccine in quality and safety has been further improved. Taking the virus vaccine as an example, this work expounded the production process of vaccine from disease material collection to inspection and determination. The quality control process of vaccine from the production to the supervision and management was also expounded.

1. Background
The discovery, use and development of vaccine is of great significance in the development of human society, and is a major breakthrough in the struggle between human beings and diseases. With the deepening and maturity of research, the use of vaccines has become an indispensable life factor to ensure the health and safety of modern human beings. From the familiar polio sugar pill to the epidemic vaccine and rabies vaccine, etc., vaccines have made outstanding contributions to the prevention, control, treatment and elimination of specific diseases. With the rising status of vaccine, the loopholes in vaccine quality supervision also arise, leading to a series of problems, which make people's health not guaranteed. From "2005 fake vaccine event in Sixian County, Anhui Province" to "2018 fake vaccine event in Shandong Province", or driven by interests, or due to production process problems, these low-quality vaccines, which should have protected human beings, may change and become "dangerous goods" that endanger human health. This work will take the virus vaccine as an example, briefly expound the basic process of modern vaccine production, and expound the quality control of vaccine.

2. Production Process of Virus Vaccine

2.1 Vaccine concepts
The vaccine is a kind of biological product for preventing, controlling and treating specific diseases, which is made of pathogenic microorganism and its metabolites. The principle of vaccine is that the pathogen (e.g., bacteria, viruses) and its metabolites are attenuated, inactivated or otherwise treated to make immune preparations that retain immunogenicity and weaken its toxicity. The immune preparation can stimulate the body to produce corresponding immune response and produce a large number of specific antibodies, so that the body can obtain the resistance to the corresponding pathogen. According to the nature of the vaccine, it can be divided into three categories: live attenuated vaccine, inactivated vaccine and genetic engineering vaccine. According to the objects of action, it can be divided into virus vaccine, bacterial vaccine, etc.

Next, the basic production process of vaccine based on virus vaccine will be introduced.
2.2 Disease material collection and virus isolation

According to the diagnosis results and the lesion location, the disease diagnosis is carried out. The appropriate method is selected to collect the disease material, and the collected disease material is preserved and inspected. The above processes shall be pollution-free, and the disease materials were treated. A large number of target viruses were obtained by inoculating the treated materials with cells or embryos. For example, Jiang Tao and others of Qingdao Agricultural University[1] isolated the virus from the suspected duck liver tissue of duck plague collected from Shandong Province. They obtained the virus isolate, inoculated duck embryo, and carried out a series of processing to obtain the target virus. For the virus with strong virulence, due to its potential safety risks, it is necessary to carry out virus reduction and culture to reduce its infectivity without affecting its immunogenicity. It includes physical inactivation (high temperature ultraviolet, etc.), chemical inactivation (guanidine nitrite, etc.), biological inactivation (non susceptible alien animals, hybrid attenuated, etc.).

2.3 Virus vaccination and culture

The virus is a small individual, a non-cellular structure composed of a nucleic acid (RNA or DNA) and a protein shell, and is not subjected to physiological activities such as reproduction without leaving the cell. Therefore, in the process of virus culture, it is necessary to carry out appropriate cell culture to obtain cells with full monolayer. The culture medium was poured out and a proper amount of virus suspension was added for adsorption treatment. After adsorption, the virus maintenance solution was added for culture, and the culture was stopped until most of the cells had pathological changes for digestion and harvest. For example, Wang Fei and Dan Xuefeng of Changchun Qijian Biological Products Co., Ltd. cultured cells in cell factory[2], and transferred them to 4 or 10 cell factories according to the concentration of 5.5×10^7 cells in each layer. 250 ml of cell growth solution (MEM + 10% calf serum + 1% glutamine + 3% sodium bicarbonate) was added in each layer, and cultured at 37°C for 72 hours. The cells grew into dense monolayer. According to 0.002≤0.004 MOI value, the virus species (varicella virus Oka strain working seed batch 47 generations) were added to the virus culture medium. After the cell factory empties the cell culture medium, the cell surface was washed with PBS solution (400mL per layer). 200 mL virus maintenance solution was added to each layer and cultured at 35°C. After culture at 35°C for 24 h, the cytopathic effect was more than 70%.

In the production of inactivated vaccine, the virus should be inactivated to make its advanced protein lose activity, but the primary structure will remain unchanged (retaining immunogenicity), such as physical inactivation (high temperature, pH, etc.), chemical inactivation (commonly used formaldehyde inactivation, etc.). For example, Baiyu, Li Min, et al., selected formaldehyde and β-propionolactone as inactivating agents[3]. Through the study and optimization of the parameters affecting the inactivation effect, the optimum inactivation process parameters were determined. In terms of safety, inactivated vaccine is relatively safe, and uninactivated vaccine may appear virulence phenomenon and has some safety risks.

2.4 Virus purification

Due to the virus often contains cell fragments, cell protein, culture medium and other impurities, it is necessary to purify the virus by ultrafiltration, centrifugation, chromatography, etc. The purpose is to test and observe whether the impurities are removed thoroughly.

Ultrafiltration is a kind of pressurized membrane separation technology (i.e., under a certain pressure, the target solution can pass through a special membrane with a certain pore diameter. The small molecules can pass through and the large molecules can not pass through, so that the large molecules can be purified). Centrifugation is divided into differential centrifugation and density gradient centrifugation. Based on the different settlement coefficient caused by the different particle size and density, the differential centrifugation method can make the particles or macromolecules with different mass settle to the bottom of the pipe in batches, so as to achieve the purpose of separation. Density gradient centrifugation is a separation method that the sample is added to the density gradient medium for centrifugation settlement or settlement balance, and the particles are distributed to a
specific position in the gradient under the action of a certain centrifugal force to form different zones.

Chromatography can be divided into adsorption chromatography, partition chromatography, ion exchange chromatography, gas chromatography, gel filtration chromatography, affinity chromatography, etc. The principles of different chromatography methods are different. For example, based on the characteristics (biomacromolecules) of specific recognition and reversible combination with some corresponding specific molecules, affinity chromatography is a chromatographic method for the separation of biological macromolecules. Ion exchange chromatography is a separation method based on different charge of protein under certain pH condition.

2.5 Virus titer determination
After obtaining the above purified virus, a series of testing procedures should also be carried out. Virus titer is a concept to measure the toxicity of the virus, which generally refers to the number of viruses with infection activity per milliliter of virus solution. The determination of virus titer can be used to determine the concentration, toxicity and titer of virus by quantitative analysis, which is an indispensable procedure for the production of vaccine. The titer of the purified virus could be determined by measuring the minimum lethal dose (MLD), the minimum infection dose (MID), the half infection dose (EID50), the half lethal dose (LD50), etc.

3. Quality Control of Viral Vaccine
On July 15, 2018, Changchun Changsheng Biotechnology Company was informed by the investigation team that it was suspected of illegally producing rabies vaccine. By July 22, the State Drug Administration ordered the company to stop production and file an investigation. The exposure of the event pushed the vaccine quality and safety issues to the focus of social discussion.

3.1 Reasons for quality control
The Regulations on the Administration of Vaccine Circulation and Vaccination have mentioned that the vaccine referred to in the regulations means a preventive biological product used for human vaccination in order to prevent and control the occurrence and prevalence of infectious diseases.

When an infectious disease strikes, the vaccine for the disease may be a life-saving medicine. When the quality of the vaccine is deteriorated, ineffective and other phenomena, there will be no corresponding immune response and immune defense line, and the results are unbearable. Even if people have not been in contact with the disease, only one prevention needle may cause the vaccine with strong virulence to cause disease, thus causing great harm. The existence of vaccine quality control is just to ensure the effectiveness and safety of vaccines used by people, so that people's health can be guaranteed. Therefore, the quality control of vaccine is indispensable.

3.2 Vaccine production control
The production and preparation of vaccine should be discussed in terms of virus types, such as attenuated vaccine or inactivated vaccine, virulence and titer requirements, etc. In terms of actual production, microbial contamination is often a major factor affecting vaccine quality. Materials, equipment, production personnel, etc., on the production line should abide by the aseptic principle. Materials should be strictly managed. Quality management records and files should be established for each raw material to avoid serious quality and safety problems caused by raw material contamination. Vaccine production equipment is an indispensable production hardware on the production line. Once microbial pollution occurs, it will affect the final quality of the production line products. The equipment in the vaccine production process, ranging from one test tube to fermentation tank and filtration system, must be strictly supervised and managed by numbering. In order to ensure the stability of the preparation process, it is also necessary to clean and sterilize in time and avoid cross contamination caused by the mixed use of reagents. In terms of production personnel, due to frequent contact with the external environment and subjective production operation, it is one of the main factors that cause microbial pollution in vaccine production. Therefore, in terms of personnel management, it
is necessary to select personnel who are healthy, disease-free and have the necessary knowledge and operation for production. Before aseptic operation, it is necessary to clean and replace the protective clothing by itself to minimize the impact on product quality. These are the key points of vaccine quality control in the production process.

3.3 Preclinical safety evaluation
The effect of human vaccine must act on the human body, so the first thing to ensure is the safety of the vaccine. After the safety is guaranteed, in order to play the required role of the vaccine, it need to test the effectiveness. As a human vaccine, neither of them leaves the test of clinical trials. However, based on uncertainty of virulence and other factors, it will bring potential risks to directly test people as subjects, and even become the pathogen of infection in healthy subjects. Therefore, before the clinical trial, it is necessary to carry out the preliminary safety test with experimental animals as subjects (i.e., preclinical safety evaluation). According to the guidelines for non clinical evaluation of vaccines published by the World Health Organization, preclinical trials are the prerequisite for vaccine development from laboratory to clinical trials.

The main contents of preclinical trials include acute toxicity test, repeated administration test, anaphylaxis test, reproductive toxicity test, etc. Acute toxicity test mainly reflects the direct damage of vaccine to the body, and offers reference for the follow-up clinical trials. Due to the dose and frequency of vaccination are relatively low, it is not possible to cause direct injury. Repetitive administration test is the core content of preclinical test, which can systematically simulate human vaccination effect and evaluate vaccine safety. Through the observation of toxic effect, injury degree and non-toxic reaction dose, it can offer reference for follow-up clinical trial design and observation index. In anaphylaxis experiment, anaphylaxis is one of the most common clinical adverse reactions of vaccines. Guinea pigs are usually used as subjects to carry out preclinical tests, and whether to carry out follow-up tests is judged according to the test results. The reproductive toxicity test mainly focuses on the vaccine to be used for women of childbearing age. Generally, sensitive animals are selected to investigate the effect of the vaccine on embryos and young children. The experiment was designed to inoculate before mating, during pregnancy and after delivery. The observation indexes should include the number of live fetuses, body weight, morphological examination, number of abortions, survival rate before weaning, etc. If necessary, the antibody level in cord or fetal blood should also be investigated to determine the level of embryo exposure.

3.4 Vaccine clinical trial
Human-subject clinical trials are essential for the application of products, so the requirements are also very strict. Here is the selection of subjects. Phase I clinical trial is a small-scale trial to preliminarily determine the safety and clinical tolerance of vaccines, usually choosing healthy adults. Phase II clinical trial is a large-scale trial to determine the safety and immunogenicity of vaccines. Phase III clinical trial is a large-scale trial to determine the efficacy and safety data of vaccines. In the phase II and phase III clinical trials, the target group of vaccine effect should be selected. When the target group is young children, the test should be conducted in the order of adults, children and infants in order to avoid accidents. The test personnel shall ensure their health, normal immune function, not vaccinated in recent period, not taking antibiotics for a long time, etc., so as to minimize the impact of personnel conditions on the test.

During the clinical trial, the quality management of each link must be strictly carried out. The site shall have the qualification approved by the relevant health department, with advanced equipment and high-quality staff. The test personnel shall receive necessary training. The test vaccine shall be strictly managed, vaccinated correctly and scientifically, and all data required for the trial shall be recorded at any time, monitored, analyzed and reviewed by the relevant staff.

3.5 Vaccine preservation and transportation control
During the preservation and transportation of the vaccine, the deterioration and inactivation of
vaccines will lead to very serious risks. Therefore, for the storage and transportation of vaccines, it is necessary to maintain the temperature control of the storage and transportation vehicles, as well as the monitoring of external temperature, environment, density and thickness of constituent materials, etc.

3.6 State supervision and management
GMP standard is a system to reduce the risk of unqualified drugs in the production process as much as possible, so as to ensure the continuous production of drugs under the specified quality. China has carried out strict management on the registration, production, storage, circulation, vaccination, etc., related to vaccines to ensure the health protection of people using qualified vaccines.

When a company applies for the production and listing of a new vaccine, it needs to have non-inferiority certificate, safety and effectiveness certificate, and support of clinical trial and research certificate to obtain the license. The production and circulation of vaccines should also be carried out under the control of the state. The national regulatory authorities will personally test and issue vaccine products in batches, so as to ensure the safety and effectiveness of vaccines and effectively monitor the circulation of vaccines. Batch issuance is mandatory inspection before the vaccine is put on the market, and unqualified products will not be allowed to be put on the market. If the quantity of circulating vaccine does not meet the quantity approved by the batch issuance work, it indicates that there are defective vaccine products.

3.7 Risk control of production process of virus vaccine
The risk management in the production process of virus vaccine is a systematic work and a highly comprehensive dynamic management activity. It involves different levels, multiple processes and multiple links. Considering that the whole biological vaccine production process needs the cooperation of all relevant departments, in the production process, the assessment team is composed of leaders and experts from process directors of all departments. Leaders and supervisors of hepatitis B vaccine are very familiar with the process, and will identify and evaluate the risks in combination with the problems encountered by employees of each department in actual work. In this paper, the production process of hepatitis B virus vaccine is taken as the main body, and risk identification analysis, evaluation and control are carried out from five aspects of "person, machine, material, method and surrounding".

![Risk Tree Of Production Process Of Virus Vaccine](image)

Figure 1. Risk tree of production process of virus vaccine

4. Value analysis of virus vaccine
The value of virus vaccine lies in the prevention and control of virus. In this paper, we choose hepatitis B virus vaccine and test its value through experiments. Hepatitis B vaccine is a kind of hepatitis B vaccine, which is isolated from the plasma of hepatitis B virus carriers and further processed in the laboratory. After vaccinating children with hepatitis B vaccine, it can improve the body's immunity and resistance, so as to ensure that the body will not be infected with hepatitis B disease and improve the health status. In this paper, 300 children who were vaccinated with hepatitis B vaccine were selected. After one month of vaccination, blood samples were taken to check the children, and the test results were analyzed. The report is as follows.
Table 1. Immune response of children with different ages

| Age          | Cases | High immune response | Low immune response | No immune response |
|--------------|-------|----------------------|---------------------|-------------------|
| Under 2 years old | 100   | 58                   | 37                  | 5                 |
| Under 4 years old | 100   | 48                   | 40                  | 12                |
| Over 4 years old | 100   | 36                   | 44                  | 20                |
| Sum          | 300   | 142                  | 121                 | 37                |

![Stacked bar chart of immune response](image)

The results showed that with the increase of age, the immune response rate of children showed a downward trend, among which the high immune response in different age groups were 58%, 48% and 36%, respectively. The low immune response and no immune response of children increased with age. At the same time, there was significant difference in the results of high immune response among three different age groups (P < 0.05). It shows that with the increase of children's age, the immune response rate begins to decline, and it is easy to cause hepatitis B infection. Therefore, we must do a good job of corresponding protection to reduce the incidence of hepatitis B infection, and promote the healthy growth of children.

5. Conclusion and Prospect
In order to ensure the quality of the vaccine, the safety and rigor of the materials, equipment, production personnel, production process shall be ensured, and the potential safety hazard caused by the error of manual operation shall be avoided. As a human vaccine, preclinical trials and clinical trials should be carried out cautiously and strictly. According to the vaccine characteristics of different age groups and different physique, the vaccine characteristics should be obtained, so as to know the root of the vaccine. Market circulation should strictly comply with national supervision and management. The transportation should ensure the correct preservation conditions of vaccines, in order to prevent the emergence of unqualified vaccines.
China's vaccine industry is very young, while it is closely related to people's health with the stage of vigorous development. In recent years, the frequent occurrence of "invalid vaccine", "fake vaccine", etc., make the relevant national departments pay more attention to the inspection and supervision of vaccines. With the continuous improvement of production and circulation supervision methods and the strengthening of law enforcement, It means that the level of vaccine production technology and quality control in China will be further improved. Under the national supervision and the efforts of relevant departments and enterprises, it is believed that a safe and effective vaccine will become the most reliable security guarantee for the people.

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