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REVIEW

Regulatory science accelerates the development of biotechnology drugs and vaccines by NIFDC

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The Chinese National Institutes for Food and Drug Control (NIFDC) is the national laboratory responsible for the quality control of pharmaceutical products. In recent years, to ensure the quality of biological products and improve the research and development (R&D) of new biological drugs and vaccines, NIFDC conducted a series of regulatory science studies on key technologies for quality control and evaluation, and established a quality control and evaluation platform for biological drugs and vaccines. These studies accelerated the R&D of the biological drugs and vaccines in China and assured their safety and efficacy. In this paper, NIFDC's duties and achievements in the biological drug and vaccine field are summarized.
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INTRODUCTION

The Chinese National Institutes for Food and Drug Control (NIFDC) is under the direct supervision of the China Food and Drug Administration. NIFDC is in charge of the quality control of pharmaceutical products and medical devices. Additionally, it is also NIFDC's responsibility to develop, distribute and manage the national reference standards. Furthermore, NIFDC takes responsibility for controlling batch releases of all vaccines in China to ensure the quality of all commercially available human vaccines.

China is a large producer of biological drugs and vaccines. However, in terms of innovation, China is still behind most advanced countries. To improve the quality standard of biological drug and vaccine, NIFDC has carried out regulatory science research that focuses on key technologies for biological drug and vaccine quality control and evaluation with funding support from the National Science and Technology Funds and other projects.[1] Such research led to the establishment of a biological drug and vaccine quality control standard and evaluation platform as well as the development of the biological drug and vaccine reference standards that were included in the 'Chinese Pharmacopoeia' part III, 2010 edition. Additionally, this research helped the launch of a number of new biological drugs and vaccines, such as hepatitis E virus, influenza A virus subtype H5N1 (H5N1) and other biopharmaceuticals as well as accelerated the development of novel vaccines, including enterovirus 71 (EV71) and H7N9 avian influenza (H7N9). This paper gives a brief introduction to the duties of NIFDC and achievements from its regulatory science research by considering the developments of the pandemic influenza vaccine and hand, foot and mouth disease (HFMD) vaccine as examples.

BACKGROUND FOR THE NIFDC

NIFDC is the statutory body for drug quality control and the highest technical arbitration institution in China. Its predecessors were the Institute for Drug and Food Control and the Institute for Biological Product Inspection, which were both established in 1950 under the Ministry of Health of the People's Republic of China. In 1961, the two merged into a new entity, National Institute for the Control of Pharmaceutical and Biological Products. In 1998, National Institute for the Control of Pharmaceutical and Biological Products became a subordinate unit of the State Drug and Food Administration. In 2010, it was renamed the National Institutes for Food and Drug Control (Figure 1). NIFDC is the Collaborating Centre on Drug Quality Assurance for the World Health Organization (WHO) and the Collaborating Centre on Biological Products for the WHO. It also has responsibility for six national key laboratories and centers, including the key laboratory center for national biotech product testing methods and standardization, Collection Center of Chinese Medicine Bacteria, National Experimental Animal Quality Inspection Center and so on. It has successfully developed the world's first Japanese encephalitis attenuated live vaccine.

NIFDC tests more than 10 000 batches of various types of drugs, biological products and medical equipments annually. It also provides more than 2900 reference substances for national drugs, biological products and medical devices. Among them, 2030 reference substances are chemical reference substances, 717 are reference herbs and 170 are bioproducts. Additionally, NIFDC has provided more than one million pieces (sets) of drug reference standards and bacteria or virus standard strains.

In addition to the statutory inspection and management of bacteria, virus seeds and standard materials, vaccine batch release is also the NIFDC's responsibility. For example, NIFDC completed vaccine batch releases for one billion doses of 55 different types of vaccines from 35 manufacturers in 2013, which ensured the quality of vaccines in China's market. Furthermore, to improve the quality of vaccines, NIFDC conducted a number of studies to enhance the vaccine quality standard and evaluation. In recent years, NIFDC actively carried out international cooperation and established collaborative relations with

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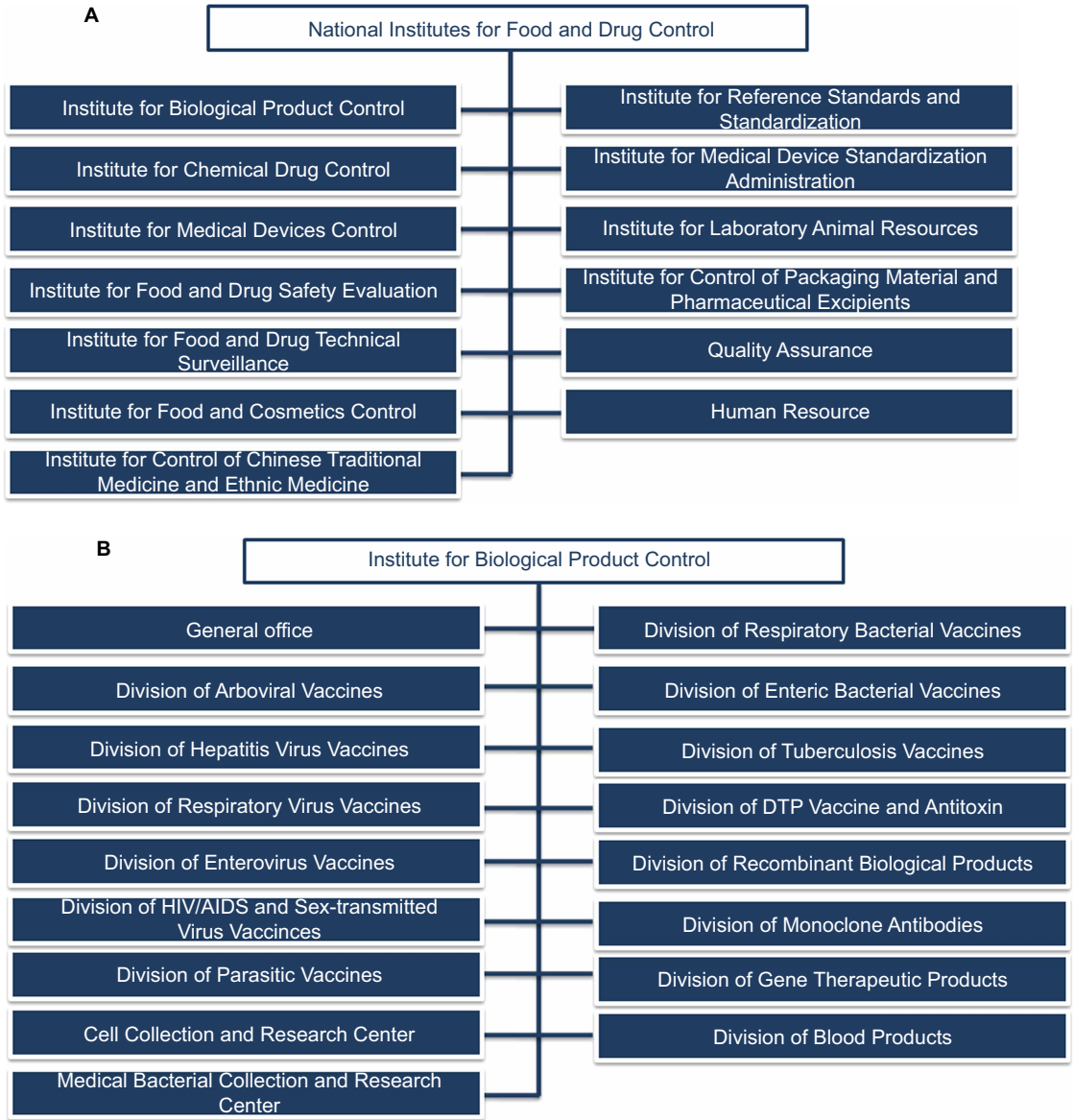

Figure 1 Organization structure. (**A**) The Divisions of NIFDC and (**B**) the Divisions of Institute for Biological Product Control. The Division of Recombinant Biological Products is in charge of establishing quality standards and evaluation methods for biotechnology drugs. The Division of Respiratory Virus Vaccines is responsible for quality control and for evaluating the pandemic influenza vaccine. The Division of Hepatitis Virus Vaccines is in charge of the quality control and evaluation of HFMD vaccines.

WHO, the National Institute for Biological Standards and Control, Centre for Vaccine Evaluation, Biologics and Genetic Therapies Directorate, Health Canada, Paul Ehrlich Institute and other biological product quality control agencies. It conducted regulatory science research and the results improved the overall quality control standards in China's vaccine and biotechnology drugs.[1] In 2013, NIFDC became a WHO Collaborating Centre. A Chinese live attenuated Japanese encephalitis vaccine was certified by WHO prequalification.

ESTABLISHING QUALITY STANDARDS AND EVALUATION METHODS FOR BIOTECHNOLOGY DRUGS

NIFDC conducted systematic standardization research to solve difficulties exist in evaluating the biological activities of class I innovative drugs. It applied, for the first time worldwide, more than 10 methods to the quality assessment of national class I innovative drugs. These methods included the endothelial cell migration method for detecting the activity of recombinant endostatin, a factor IX gene knockout mouse method for determining the activity of gene therapy drug AAV2/F IX and the enzyme-linked immunosorbent assay for detecting the activity of recombinant neuregulin kinase receptor activation. After a thorough analysis of the signal transduction mechanism, NIFDC first applied transgenic cell technology to establish the luciferase reporter gene approach by transferring drug receptors or reporter genes into cells to determine the interferon activity. This approach overcame a series of shortcomings of the previously internationally adopted method of viral suppression and improved the measurement efficiency by three times.[2] Based on the same principles and techniques, NIFDC, for the first time, established the second messenger cGMP competing assay for the determination of brain natriuretic peptide activity. Compared to the traditional rabbit aortic

strip method, this new method can lower the coefficient of variation from 66% to 12%.[3] The technology has been incorporated into the WHO Collaborating Centre promotion program.

Due to the characteristics of biological macromolecules and a lack of international standards, NIFDC successfully developed 10 much-needed standards for the research and development (R&D) and production of biomedicine, which solved the problem of evaluating the critical quality attributes and improved the overall quality of those biological drugs in China. Among those standards, the DNA detection of residual host cells is an important safety indicator for lowering the potential carcinogenic risk. Because of a lack of detection standards domestically and abroad, NIFDC, for the first time, successfully developed the quantitative national standards[4,5] for Vero cell DNA (2011 National Health standard 0045) and *Escherichia coli* DNA (2012 National Health Standard 0026), which improved the accuracy and comparability of DNA measurements. Those standards have been widely used in the production and quality control of biological drugs for rabies, Japanese encephalitis, polio, interferon, granulocyte-colony-stimulating factor and more. Albumin is used as a detection standard for measuring the protein content of biological drugs. NIFDC proposed an innovative idea of biopharmaceutical homogeneous standard calibration and assignment as well as developed a national standard to detect the content of four types of homogeneous proteins, including the granulocyte colony-stimulating factor.[6] This new standard overcomes the system deviation caused by the non-homogeneity of the previous standard and test sample, which was included in the 2010 edition of 'Chinese Pharmacopoeia'. NIFDC was invited to present the results of this study on the 'quality assessment seminar of WHO therapeutic biological drugs'.

The aforementioned methods and standards research have led to the development of 34 biological drug quality standards that were included in the 'Chinese Pharmacopoeia', and these were applied to the quality evaluation of more than 40 class I drugs in China. Those standards ensured the safety and efficacy of innovative biological drugs in China.

ESTABLISHING QUALITY CONTROL AND AN EVALUATION SYSTEM FOR THE R&D OF THE PANDEMIC INFLUENZA VACCINE

Periodic outbreaks of pandemic influenza pose a great threat to human health and social development. Although the seasonal vaccine production process might be used for the production of pandemic influenza vaccine, there are challenges in the development and evaluation of the pandemic influenza vaccine. Since 2004, with the support of the national special fund, NIFDC created the key technology system for the development and evaluation of the vaccine and has successfully applied this system to the development of H5N1 and H1N1 human avian influenza vaccines.

With the reality that the virus seed of the pandemic influenza vaccine is often prepared with the reverse genetics method, NIFDC introduced the gene sequencing method for the evaluation of mutation and genetic stability of the virus seed stocks. This new method ensured the quality of the vaccine from the source, enabling manufacturers to promptly start mass production.[7,8] The vaccine development was usually delayed after the manufacturers had the bulks because the WHO could not provide quantitative reference materials in a timely fashion and the vaccines could not be quantified. To solve this problem, NIFDC carried out a prospective study and established an improved sodium dodecyl sulfate–polyacrylamide gelelectrophoresis method for the determination of hemagglutinin. This method has been fully validated. During the development of the H1N1 influenza vaccine in 2009, NIFDC prepared the world's first H1N1 influenza antigen national reference using this method. This reference was successfully applied to vaccine development and quality control as well as shortened China's overall development process of this vaccine by one month.[9,10] NIFDC also collaborated with foreign agencies to establish and validate, for the first time, a universal antibody and the corresponding quantitative detection technique, which can be used to measure the hemagglutinin content from different types of influenza viruses. This antibody and new technique improved the technical support capabilities in developing new vaccines for the influenza pandemic variants in the future.[11,12]

Due to the special nature of the pandemic influenza vaccine, an adjuvant is often needed in the finished product to enhance the immunogenicity. NIFDC has also established a rapid and accurate *in vitro* detection method for vaccines with adjuvants to replace the *in vivo* animal test, which was time-consuming and had low efficacy and poor accuracy. The testing period has been shortened from 30 days to 2 days. This method has been used in the research, development and evaluation of adjuvant vaccines for human bird flu (H5N1) and H1N1 influenza, so that clinical trials on vaccines with adjuvants and conventional vaccines could be carried out simultaneously.[13]

During the clinical trials, volunteers' serum samples were collected to detect the antibody titers after inoculation and to determine the efficacy of the vaccine. Therefore, a reliable serological result determines the success of the clinical trial. NIFDC established a standardized clinical serological evaluation method for the flu vaccine. In international collaborative studies, the accuracy, repeatability and other indicators for this method were consistent with the methods used by other international advanced laboratories.[14] During the H1N1 influenza vaccine clinical trials, NIFDC tested more than 40 000 serum samples, which provided the most critical efficacy data for the vaccine's evaluation and approval. The relevant results have been published in major international journals.[15–19]

ESTABLISHING QUALITY CONTROL AND EVALUATION TECHNIQUES FOR ACCELERATING THE PROCESS OF THE R&D OF THE HFMD VACCINE

In recent years, the HFMD incidence in the Western Pacific region has gradually increased, in terms of the frequency, severity and number of reported deaths. Approximately 9.04 million cases, including 2712 deaths, were reported in China during the period 2008–2013. HFMD has become a serious public health problem in this area. HFMD pathogens are mainly EV71 and coxsackievirus A 16 (CA16). Due to a lack of effective drug treatment, a number of agencies in mainland China, Taiwan and Singapore have started related vaccine research and development.

To accelerate the HFMD vaccine development process, NIFDC started conducting studies to solve technical bottlenecks, such as selecting HFMD vaccine virus seed, developing reference standard and evaluating immunogenicity, in 2008. Based on the studies of the biological and antigenic characteristics and total genome sequence comparison of EV71 vaccine candidate strains in mainland China, vaccine strains were confirmed. Based on three EV71 vaccine clinical trials, cross-protection studies were carried out to investigate the cross-protective effect of the vaccine in Chinese children. The results show that EV71 vaccines from the C4 genotype strain have a widely protective effect on the epidemic strains in other countries. Such work provides the basis for determining the EV71 vaccine strains for research organizations and the applicability of the vaccine.[20,21] To

further ensure the EV71 vaccine immunogenicity reliability and comparability, national EV71 quantitative standards for the vaccine antigen and neutralizing antibody were established (2010 NOs 0023 and 0024). These standards were used for the accurate quantification and comparison of vaccines from different companies that were made from different strains with different cellular substrates and production processes.[22] According to structural biology studies, the crystal structures were revealed. EV71 has two different crystal structures, 'empty' and 'full' structures. CA16 has three different crystal structures. These results provide new ideas for the process improvement and quality research of similar vaccines.[23,24]

To ensure the accuracy and reliability of vaccine clinical trial results, NIFDC carried out testing and evaluation studies for those vaccine samples.[25–27] The results from the completed EV71vaccine phase III clinical trials showed that the protection rate of vaccines developed by three Chinese companies was more than 80% against EV71-related diseases and was more than 90% against HFMD caused by EV71.[28–30] Professor Peter C McMinn in Australia commented that 'a priceless gift will have been given to the children of the Asia–Pacific region and to the rest of the world'.[31]

To promote the R&D of CA16 vaccine and EV71–CA16 bivalent vaccine, NIFDC established a neonatal mouse model for CA16 vaccine evaluation[32] and successfully developed the national reference standard for the CA16 neutralizing antibody assay (2014 NO 0029). Currently, some Chinese companies have completed the preclinical EV71–CA16 bivalent vaccine research and have applied for clinical trials.

SUMMARY

By conducting vaccine regulatory science research, NIFDC has improved the vaccine quality standard and evaluation method, ensured the safety and efficacy of vaccines on the market and promoted the development of new biotechnology drugs and vaccines. China was the first country to commercially launch the pandemic vaccine, and the results from the EV71 vaccine phase III clinical trial demonstrated the safety and protective effect of this vaccine. NIFDC's related research results were recognized as examples of regulatory science.[1] In 2013, NIFDC became a WHO Collaborating Centre, and in the same year, China's live attenuated Japanese encephalitis vaccine was certified by WHO prequalification, which was a contribution to the world from the Chinese biotechnology drug and vaccine industry. Currently, NIFDC has been improving its regulatory science research to enhance the biological drugs and vaccines through active international and domestic collaborations.

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