

**(Attachment)**

**Guidelines for Nonclinical Studies of Vaccines for Infectious Disease Prevention**

Revised on March 27, 2024

**1. Introduction**

**1.1. Background**

With recent advances in biotechnology and immunology, the development of novel vaccines and improvements to already-approved vaccines have been ongoing. However, due to the diversity and species specificity of vaccines, uniform criteria for evaluating their safety and pharmacological effects do not exist. Therefore, to characterize the safety and pharmacological effects of novel vaccines, nonclinical evaluation items and content need to be determined based on current scientific standards.

Nonclinical studies of vaccines are methods used to predict their efficacy and safety in humans and play a critical role in transitioning vaccine development from the preclinical to the clinical stage.

**1.2. Purpose**

Nonclinical studies of vaccines are conducted to characterize vaccine products (including for the safety and pharmacological effects). These Guidelines provide general principles for planning nonclinical studies of vaccines.

The primary objectives of conducting nonclinical studies are:

1. To evaluate pharmacological effects,
2. To identify potential target organs/tissues for toxicity,
3. To evaluate safety at dose levels for humans,
4. To assess whether observed toxicity findings are reversible, and
5. To determine safety endpoints for clinical monitoring.

**1.3. Scope**

These Guidelines apply to the development of vaccines for the prevention of the onset of infectious diseases or the prevention of infections (hereinafter referred to as 'infectious disease prevention'). These Guidelines do not apply to the development of "therapeutic vaccines (such as antitumor vaccines [cancer vaccines]). Vaccines for infectious disease prevention covered by these Guidelines are those that induce specific immunity against pathogenic microorganisms and others, such as:

1. Vaccines containing, as active ingredients, chemically or physically inactivated microorganisms, which maintain their immunogenicity (such as Japanese encephalitis vaccine),

2. Vaccines containing as active ingredients either microorganisms antigenically similar to human pathogens, or attenuated microorganisms that maintain appropriate immunogenicity (such as measles vaccine, BCG vaccine, etc.),
3. Vaccines containing as active ingredients either antigens extracted from pathogenic microorganisms or toxoids that have been obtained by inactivating toxins produced by pathogenic microorganisms (such as influenza HA vaccine, pertussis vaccine, diphtheria toxoid, tetanus toxoid, etc.),
4. Vaccines containing as active ingredients antigens obtained through genetic engineering techniques, or these antigens aggregated, polymerized, or conjugated to carriers (such as hepatitis B vaccine, pneumococcal conjugate vaccine, etc.),
5. Vaccines that are genetically engineered viruses or bacteria, and
6. Vaccines containing expression plasmid DNA as active ingredients.

Note that if specific guidelines have been issued separately, they must be separately addressed.

In the development of novel vaccines not included in the above, it is advisable to start consultations with regulatory authorities from an early stage of development after summarizing available findings.

## **2. General Principles**

Nonclinical studies of vaccines are conducted to characterize their pharmacological effects and safety. Vaccines are associated with potential safety concerns such as inherent systemic toxicity, excessive local reactions, adverse immune reactions such as unintended sensitization, and in some cases reproductive and developmental toxicity, toxicity from impurities, and interactions of ingredients present in the product. Therefore, nonclinical studies should be conducted for novel vaccines. The safety of novel adjuvants and novel excipients, if any, should also be evaluated. However, when there is a scientifically justifiable reason, nonclinical studies required for other novel vaccines are not always required, such as when a novel vaccine is a combined vaccine consisting only of active vaccine ingredients already approved in Japan or when the composition and pharmacological effects of a novel vaccine are similar to those of an approved vaccine for which there are many clinical use results and its safety has been established.

### **2.1. Study Design**

The necessity of nonclinical studies, study type, animal species selection, and study design should be considered based on scientific evidence and the specific characteristics of each vaccine. Nonclinical safety studies are required to be conducted in compliance with the Ministerial Ordinance on Good Laboratory Practice (GLP). If the study cannot be conducted in compliance with GLP, it is required to specify the non-GLP parts and explain their impact on the safety evaluation.

In designing nonclinical studies in animals, the animal species, dosages, route of administration, study duration, assessments (e.g., clinical observations, body weight, hematology, urinalysis, necropsy, histopathology), and timing of assessments should be determined. As for the dosages in nonclinical studies, the intended administration method in clinical practice should be considered to determine the doses, dosing intervals, dosing frequencies, and dosing durations.

## **2.2. Selection of animal species/models**

When selecting animal species for nonclinical studies of a vaccine, usually at least one species that exhibits immune response to the active ingredient of the vaccine should be used. It is not always necessary to select a non-human primate.

## **2.3. Test article**

The test article used in nonclinical studies of a vaccine should appropriately reflect the characteristics (such as composition, dosage form, and manufacturing method) that may affect the efficacy and safety of the product for clinical use.

## **2.4. Route of administration**

In principle, the route of administration should follow the intended clinical route. In nonclinical safety studies, systemic safety can be evaluated even if the test article is administered via a route other than the intended clinical route of administration, provided that systemic immune responses (e.g., antibody production) similar to those with the clinical route are induced. However, even in such cases, an evaluation of local tolerance using the intended clinical route is required.

## **3. Pharmacology**

Nonclinical studies to assess pharmacological actions of vaccines should adopt testing methods expected to have sensitivity and specificity suited for the purpose of each study.

### **3.1. Immunogenicity**

Studies to evaluate the immunogenicity of vaccines include assessment of the production level of the antibody that is expected to be related to the protection against infections or the prevention of disease onset, the class and subclass of the antibody produced, and cytokine production affecting the cellular immunity and immune system.

### **3.2. Protection against infections**

If animal models reflecting the infections/diseases of interest in humans are available, it is desirable to use such a model and assess the protection against the infections/diseases caused by the pathogenic microorganism targeted by the vaccine as an endpoint.

### **3.3. Safety Pharmacology**

When evaluating the nonclinical safety of vaccines, usually, the effects on major physiological functions (central nervous system, respiratory system, and cardiovascular system) can be assessed in nonclinical toxicity studies through the observations and tests in those studies. A separate safety pharmacology study should be considered if these assessments raise safety concerns for major physiological functions.

## **4. Pharmacokinetics**

Pharmacokinetics studies are usually not required for vaccines.

However, for vaccines containing expression plasmid DNA as an active ingredient, biodistribution must be, in principle, investigated before starting clinical studies.

For novel live attenuated vaccines, investigation of shedding is helpful in designing clinical shedding studies. When relevant findings in animals have been obtained in nonclinical pharmacological studies, etc. of the vaccine or sufficient findings concerning infection of the wild-type virus in humans have been obtained, no separate shedding study of the vaccine is needed for this investigation.

## **5. Toxicology**

### **5.1. Single-Dose Toxicity**

Although acute toxicity should be assessed, it can usually be done using the findings obtained after the initial doses in repeated-dose toxicity studies.

### **5.2. Repeat-Dose Toxicity**

Generally, the number of doses administered in a repeat-dose toxicity study should exceed the planned number of vaccinations for clinical use. The doses should be approximately the same as the one dose for clinical use. If, however, it is physically unfeasible to administer the same dose as that in humans, it is necessary to use at least a dose (mg/kg or mL/kg) that exceeds a weight-based dose in humans (mg/kg or mL/kg). If such a dose selection is considered not appropriate from a technical and animal welfare point of view, the maximum feasible dose in the animal species used should be administered. If any toxicologically significant findings are observed at these doses, the need for conducting a nonclinical safety evaluation at lower doses should be considered to ensure clinical safety. When observing clinical signs, attention should also be paid to the condition of the injection site, hypersensitivity reaction, etc. In histopathology, at least major organs (brain, lungs, heart, kidneys, liver, and reproductive organs) as well as major immune organs (thymus, spleen, bone marrow, and regional lymph nodes at the injection site) should be examined. Blood testing should also be conducted. If any adverse changes are observed in these tests, the reversibility of the changes should be examined.

### **5.3. Reproductive and Developmental Toxicity**

Among the effects of reproductive and developmental toxicity, those on fertility can be assessed as part of the histopathological examinations of the reproductive organs in repeated-dose toxicity studies. The need for studies on embryo-fetal development and studies on pre- and postnatal development, including maternal function should be determined based on the intended vaccine recipients in clinical use. When conducting these studies, endpoints in the period from reproduction stage C (implantation to hard palate closure) to stage E (birth to weaning) are typically evaluated using a single animal species. If appropriate contraceptive measures are ensured in clinical studies, reproductive and developmental toxicity studies can be conducted by the time of a marketing application. However, if there is any concern about reproductive and developmental toxicity, this evaluation should be conducted by the time of initiating large-scale clinical trials.

### **5.4. Genotoxicity**

Genotoxicity studies are generally not required for vaccines.

### **5.5. Carcinogenicity**

Carcinogenicity studies are generally not required for vaccines.

### **5.6. Local Tolerance**

Local tolerance may be evaluated as part of the single-dose or repeat-dose toxicity studies; if this is the case, separate local tolerance studies are not necessarily required.

### **5.7. Toxicokinetics**

In general, vaccines do not require a toxicokinetic evaluation.

## **6. Special Considerations**

### **6.1. Adjuvants**

When a novel adjuvant is used for a vaccine product, the safety of the adjuvant should be evaluated in studies that use the vaccine product, etc. with special attention being paid to reactions such as local reactions and hypersensitivity. For vaccines with a new combination of an adjuvant contained in an approved vaccine and an approved active vaccine ingredient, additional nonclinical safety assessments, including local reactions, are required if there is any concern about new toxicity. In addition, when a vaccine product contains a novel adjuvant, a biodistribution study for the novel adjuvant may be needed.

### **6.2. Excipients (excluding adjuvants)**

When a vaccine product contains new excipients (stabilizers, solubilizers, preservatives, pH regulators, etc.) that have not been previously used, the safety of the excipients needs to be

evaluated in studies that use the vaccine product, etc.

### **6.3. Combined vaccines**

For a novel combined vaccine (including those combining approved active vaccine ingredients), interactions (such as interference and suppression) between the combined active vaccine ingredients may occur. Therefore, the possibility of enhancement or attenuation of the immune responses (pharmacological effects and effects on safety) associated with the combination should be investigated.

### **Glossary**

#### **Adjuvant**

A pharmaceutical aid to facilitate immune response. A substance that, when co-administered with an antigen *in vivo*, nonspecifically enhances the immune response to the antigen.

#### **Immunogenicity**

Ability of a vaccine to induce an immune response (humoral and cell-mediated immunity).

#### **Combined vaccine**

A vaccine containing multiple active vaccine ingredients against multiple infectious diseases.