

Report on the Deliberation Results

September 18, 2007

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name] Adsorbed Influenza Vaccine (H5N1) “HOKKEN”
[Non-proprietary name] Adsorbed Influenza Vaccine (H5N1)
[Applicant] The Kitasato Institute
[Date of application] January 30, 2007

[Results of deliberation]

In the meeting held on August 31, 2007, the Second Committee on New Drugs concluded that the product may be approved and that this result was to be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

It was decided that the product is classified as a biological product, its re-examination period is 10 years, and both the drug substance and the drug product are classified as powerful drugs.

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency, the Japanese text shall prevail. PMDA shall not be responsible for any consequence resulting from use of this English version.

Review Report

August 15, 2007

Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “HOKKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Kitasato Institute
[Date of application]	January 30, 2007
[Application classification]	1-(1) Drugs with new active ingredients
[Dosage form/Strength]	Injectable suspension with the following constituents per mL: 30 µg (HA content) of a strain of inactivated pandemic influenza virus as an active ingredient, 0.3 mg (on an aluminum content basis) of aluminum hydroxide gel as an adjuvant, and 10 µg of thimerosal as a preservative
[Items warranting special mention]	· Minimum Requirements for Biological Products (draft) “Adsorbed Influenza Vaccine” has been submitted. · Orphan drug (Designated on: June 9, 2006)
[Reviewing office]	Office of Biological Products

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Review Results

August 15, 2007

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “HOKKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Kitasato Institute
[Date of application]	January 30, 2007 (Application for marketing approval)
[Results of review]	

From the submitted data, it was judged that Adsorbed Influenza Vaccine (H5N1) is expected to have a protective effect against pandemic influenza (H5N1) infection and also to prevent symptoms becoming more severe, and that there should be no serious problem in safety.

In regard to the efficacy, the results of clinical studies in Japan showed inoculation with Adsorbed Influenza Vaccine (H5N1) induced antibody production ; indicating that the immunogenicity of the product. In addition, the results of challenge tests in mice demonstrated that Adsorbed Influenza Vaccine (H5N1) prevented the onset of disease after infection with a virulent strain of influenza virus (H5N1). These results suggest that Adsorbed Pandemic Influenza Vaccine may be expected to have a protective effect against pandemic influenza (H5N1) infection and to prevent symptoms becoming more severe. In regard to the safety, there were no serious adverse reactions. Taking into account the seriousness of the target disease, there may be no major problems discouraging approval of the application. However, more complete information should be provided on the use of Adsorbed Influenza Vaccine (H5N1).

Based on the results of the regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product will be approved for the following indications and dosage and administration.

[Indications]	Prophylaxis of pandemic influenza (H5N1).
[Dosage and administration]	The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses.

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Review Report (1)

July 3, 2007

I. Product Submitted for Registration

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “HOKKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Kitasato Institute
[Date of application]	January 30, 2007 (Application for marketing approval)
[Dosage form/Strength]	Injectable suspension containing the following constituents per mL: 10 µg or 30 µg (HA content) of a strain of inactivated pandemic influenza virus as the active ingredient, 0.3 mg (on an aluminum content basis) of aluminum hydroxide gel as an adjuvant, and 10 µg of thimerosal as a preservative
[Proposed indications]	Prophylaxis of pandemic influenza
[Proposed dosage and administration]	The usual dosage is an injection of 0.5 mL per dose administered intramuscularly or subcutaneously, or administered a second time after an interval of about 3 weeks.
[Items warranting special mention]	<ul style="list-style-type: none">· Minimum Requirements for Biological Products (draft) “Adsorbed Influenza Vaccine” has been submitted.· Orphan drug (Designated on: June 9, 2006)

II. Summary of the Submitted Data and Outline of the Review by the Pharmaceuticals and Medical Devices Agency (PMDA)

A summary of the documents submitted by the applicant and the answers to the questions raised by the Pharmaceuticals and Medical Devices Agency (hereinafter referred to as PMDA) are as follows.

1. Origin or background of discovery and usage conditions in foreign countries, etc.

Influenza is an acute respiratory disease caused by infection with influenza viruses, which belong to the family *Orthomyxoviridae*. Influenza viruses are classified by their serotypes into influenza type A, type B, and type C. Type A influenza viruses are further classified into subtypes (H1 to H16 and N1 to N9) according to differences in the antigenicity of the viral surface hemagglutinin (HA) and neuraminidase (NA). Host animals for the influenza A viruses differ depending on the subtype of the viruses, and include humans, birds, swine, and horses; however, all the subtypes have been isolated from birds. Subtypes H1N1 and H3N2 of influenza A virus have been identified as the causes of repeated epidemics in humans. The repeated epidemics of influenza each year are caused by the gradual change in the

antigenicity of the virus subtypes every year due to antigenic drift, and therefore, by the inability of the influenza-specific antibodies already existing in humans to completely neutralize the viruses. On the other hand, due to antigenic shift, the type A influenza viruses may mutate into new subtypes of viruses having different antigenicity and species specificity. There is concern that if this influenza virus becomes infectious to humans, the immunity already acquired in humans may not provide protection against infection, and eventually human-to-human infection with the virus may cause a worldwide pandemic. As a matter of fact, pandemics of influenza caused by pandemic strains of influenza viruses have already occurred 3 times in the 20th century: Spanish influenza (subtype H1N1) in 1918, Asian influenza (subtype H2N2) in 1957, and Hong Kong influenza (subtype H3N2) in 1968. These pandemics caused numerous health hazards, reportedly accompanied by reduction in economic and social activities.

Pandemic influenza is defined as “influenza epidemic in human beings caused by infection with an HA and/or NA virus subtype that has not infected humans for decades” in the “Report on Pandemic Influenza Preparedness” (August, 2004). Theoretically, it is possible for any influenza A virus subtypes other than H1N1 and H3N2 to cause pandemic influenza. Since the time human infection with a highly pathogenic H5N1 avian influenza strain was reported in Hong Kong in 1997, infections of humans with highly pathogenic H5N1 avian influenza strain have been reported from all over the world (191 deaths out of 315 cases of infection, as of June 25, 2007; www.who.int/csr/disease/avian_influenza/country/en/index.html). In the human cases of influenza confirmed to be caused by this virus until date, the disease has been observed to be very serious, in addition to a high mortality rate, being frequently complicated by systemic infection, bleeding tendency, multi-organ failure, and cytokine storm which are beyond the realm of influenza routine clinical practice. Therefore, pandemic influenza caused by this virus poses a grave threat never faced before by human beings. The H5N1 influenza virus is considered as a highly likely cause of pandemic influenza, and at present, H5N1 influenza is under surveillance for the trend of its occurrence by the WHO with extensive international cooperation. Also, pandemic influenza preparedness plans are being developed internationally.

In Japan, “Report on Pandemic Influenza Preparedness” was published on October 24, 1997, by the “Investigative Committee for Pandemic Influenza Preparedness” established in the then Ministry of Health and Welfare in May 1997. This report pointed out the necessity of development of countermeasures against seasonal influenza, which is the basis of countermeasures against pandemic influenza, of establishment of a system for manufacture of influenza vaccines, and of improvement and development of the manufacturing technology to allow an adequate response to a potential increase in demand in the event of a pandemic. Consolidation of the system has been promoted, including stipulation of “Basic Guidelines for Health Crisis Control” and establishment of “Coordination Meeting for Health Crisis Control.” The “Law concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections (hereinafter referred to as the “Infectious Disease Control Law”) was enforced in

1999. Based on this law, “Guidelines for Prevention of Specified Infectious Diseases Related to Influenza” was stipulated and “Liaison Meeting for Comprehensive Countermeasures against Influenza” was established as a sub-committee of “Coordination Meeting for Health Crisis Control.” In addition, the “Report on Pandemic Influenza Preparedness” was prepared in August 2004 by the “Investigative Subcommittee for Pandemic Influenza Preparedness” and published in October 2003 in *Infectious Diseases Committee of Health Sciences Council*. In this report, a direction for the development of a new vaccine against pandemic influenza was proposed. This included: (1) the necessity of development of a vaccine containing an adjuvant (auxiliary immune substance) based on the investigation conducted by Tashiro et al., as described later, (2) pandemic influenza vaccine development based on the manufacturing method of a “mock-up” vaccine*¹, and (3) obtainment of its marketing approval in accordance with the Pharmaceutical Affairs Law.

* 1 Pandemic influenza vaccine development based on the manufacturing method of a “mock-up” vaccine

When pandemic influenza occurs, it is not realistic to start development of the vaccine using the pandemic influenza virus strain after it has actually become established in the community, considering the times of development and manufacturing. Therefore, it would be desirable to obtain marketing approval for a mock-up vaccine prepared using a model virus instead of the pandemic influenza virus. If and when pandemic influenza occurs, the pandemic influenza vaccine will be manufactured using the pandemic influenza virus strain and supplied promptly according to the approved manufacturing method of the mock-up vaccine.

Based on the “WHO Global Influenza Preparedness Plan” published by the WHO in May 2005, each country established an action plan to be implemented at the time of occurrence, if it occurs, of pandemic influenza. In Japan, the “Pandemic Influenza Preparedness Action Plan” (hereinafter referred to as “Action Plan”) was formulated in November 2005, with the Ministry of Health, Labour and Welfare (MHLW) playing a central role, and collaboration and cooperation of other ministries and agencies concerned were ensured. This Action Plan will be applied, in principle, for the use of the pandemic influenza vaccine.

In response to the “Report on Pandemic Influenza Preparedness” prepared in August 2004, prior to the development of Adsorbed Influenza Vaccine (H5N1) [hereinafter referred to as the “H5N1 Vaccine”], the development policy for the pandemic influenza vaccine was confirmed at a meeting of MHLW, PMDA, the National Institute of Infectious Diseases, and ■ companies manufacturing influenza vaccine, which are members of Saikin Seizai Kyoukai, held in August 2004. In the Health and Labour Sciences Research conducted from 2001 to 2002 by Tashiro et al., a strain isolated from the highly pathogenic avian influenza, Hong Kong156/97 (H5N1), that was isolated in Hong Kong in 1997 was attenuated by the reverse genetics approach, and an HA vaccine and whole virus vaccine were prepared experimentally using the attenuated strain, by an already approved manufacturing method (neither of the vaccines contained an adjuvant). In a clinical pharmacology study, no significant increase of the antibody titers

was observed after inoculation of animals with these vaccines, although slightly higher neutralizing antibody titers were obtained after inoculation of whole virus vaccine than after that of the HA vaccine (<http://mhlw-grants.niph.go.jp/niph/search/NIST00.do>, Study on safety and efficacy of inactivated influenza H5N1 whole virus vaccine, 2002). Based on these results, it was decided to develop the whole virus vaccine, which shows higher immunogenicity, with the addition of the aluminum adjuvant that has been added to vaccines as an immune-auxiliary substance. A strain of NIBRG-14 obtained by attenuation, using the reverse genetics approach, of A/Viet Nam/1194/2004 (H5N1) isolated in Viet Nam in 2004 by the National Institute for Biological Standards and Control (NIBSC) of the UK was to be used as the strain for the manufacture of the mock-up vaccine.

In the Notification No. 0331007 issued by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated March 31, 2006, drugs designated as orphan drugs were defined as “pharmaceuticals used for the prevention of infectious diseases.” Among these drugs, vaccines to be designated as orphan drugs are defined as “vaccines used for the prevention of new infectious diseases which might occur due to genetic mutation or recur and of which possible occurrence or recurrence cannot be excluded. Also, any of the diseases may have a critical impact on the life and health of people once it occurs, the time of its occurrence and the magnitude of the epidemic are unknown, and there is no occurrence of the disease at the time of application for designation.” On the basis of the H5N1 Vaccine falling under the above category, orphan drug designation for the Vaccine H5N1 was applied to MHLW on April 13, 2006, and the designation was granted to the drug product (Designation No. 185) on June 9, 2006, for the proposed indication of “prophylaxis of influenza (H5N1).”

2. Data relating to quality

The development of the H5N1 Vaccine was urgently sought as a countermeasure against pandemic influenza, as described in the “Report on Pandemic Influenza Preparedness” in August 2004. Therefore, no sufficient data had been acquired when the application was submitted. Furthermore, it was proposed, after submission of the application, that a stabilizer be added in the manufacturing process of the bulk. Thus, although such change in the manufacturing process necessitated additional tests, the newly available data was not enough. Taking into account the information obtained from repeated inquiries to the applicant and additional data obtained after the application, and on the basis of the revised document submitted in ■■■, 200■, the data relating to the quality of the H5N1 Vaccine is summarized below. PMDA is requesting the applicant to submit further additional data and information.

Summary of the submitted data

The H5N1 Vaccine is a vaccine. Pandemic influenza virus to be used as the seed is propagated in embryonated eggs, purified virions are inactivated using formalin, and then aluminum hydroxide gel is added as an adjuvant to the inactivated virus.

(1) Drug Substance

1) Manufacturing method

a. Origin and control of seed

NIBRG-14 Vero1/E2, an attenuated virus strain of A/Viet Nam/1194/2004(H5N1), was developed by the reverse genetics approach at the National Institute for Biological Standards and Control (NIBSC), UK. NIBRG-14 Vero1/E2/E1, the first subculture of NIBRG-14 Vero1/E2 in special pathogen-free (SPF) embryonated eggs, was supplied from the National Institute of Infectious Diseases, which served as the master seed (MS). It was further propagated in SPF embryonated eggs by the applicant to obtain the NIBRG-14 Vero1/E2/E2 to be used as the master seed and the working seed (MS/WS). For the MS and the MS/WS of the NIBRG-14 strain, determination of hemagglutination (HA) titer (HA reaction), determination of infectivity titer (in embryonated eggs), sterility test, and mycoplasma test were performed as in-process control tests, and antigenicity test (the test for identifying the base sequence of the HA gene, the hemagglutination inhibition [HI] test) was performed as the characterization test.

The seeds were stored at -70°C or lower. The HA titer and infectivity titer were determined after 25 months of storage of the MS. The results showed no significant changes in the MS under this temperature condition.

The MS is to be not renewed. However, a new WS is to be prepared by further subculturing the MS/WS culture, namely, by making the 3rd subculture of NIBRG-14 Vero1/E2, according to the standard for the renewal of the MS/WS at the time of application, i.e., when the number of remaining MS/WS decreases to 10, when contamination by bacteria or fungi is detected, or when changes in the product clearly originates from the seed.

The MS was directly used in the manufacture of the bulk for lot number series 04 and 05, including lots used for the non-clinical and clinical studies.

b. Manufacturing method

Virus incubation

Thaw the seed and dilute with buffer containing █ μg/mL each of gentamicin sulfate and kanamycin sulfate to prepare a virus suspension to be inoculated. Inoculate the suspension into █ embryonated eggs aged 11 or 12 days and incubate at █°C ± █°C for █ ± █ hours. After the completion of incubation, cool the inoculated eggs at █°C ± █°C for █ to █ hours, collect the allantoic fluid (█-█ L), and then serve this solution as the virus suspension.

Concentration process and purification process

Add █ solution and █ solution to the virus suspension, centrifuge and collect the supernatant,

and perform sterile filtration with a filter with a pore size of █ μm. Concentrate this solution using the ultrafiltration membrane (molecular weight cutoff, █0000) to make █ to █ L. It is possible to keep the concentrated virus suspension thus obtained in a cool and dark place (█°C ± █°C) for up to █ hours. Fractionate the concentrated virus suspension by sucrose density-gradient centrifugation using a continuous ultracentrifuge, collect the virus fraction, and dilute to █ L with buffer. Purify this solution by █ centrifugation, dilute the obtained virus █ solution to █ L with buffer, dilute it further to reach a protein concentration of █ to █ μg/mL, and serve this solution as the purified virus suspension prior to virus inactivation. The test for determining the percent HA content (using SDS-polyacrylamide gel electrophoresis [SDS-PAGE]) is defined as an in-process control test for the pre-inactivation purified virus suspension.

Inactivation process

Add formalin solution to the pre-inactivation purified virus suspension to a final concentration of █% (w/v), and inactivate at █°C ± █°C. Perform the inactivation test as an in-process control test █ weeks after the initiation of inactivation treatment, in order to confirm the completion of virus inactivation. When complete inactivation is not confirmed, allow to stand still at █°C ± █°C again, and perform the inactivation test every █ weeks until virus inactivation is confirmed. Use the completely inactivated virus suspension as the inactivated virus suspension.

Desugaring process and filtration process

Dialyze (desugar) the inactivated virus suspension using an ultrafiltration membrane (molecular weight cutoff, █0000), dilute with phosphate buffered saline (PBS) to reach a protein concentration of █ to █ μg/mL, and perform the test for measuring the sugar concentration as an in-process control test. To this solution, add formalin as a stabilizer to a final concentration of █% (v/v). It is possible to keep the inactivated virus suspension, which is obtained after desugaring, at █°C ± █°C for up to █ hours. Perform sterile filtration of the inactivated virus suspension using a filter (pore size, █ μm) after desugaring. The integrity test of the filter is defined as an in-process control test. Add thimerosal to the filtrate to a final concentration of █% (w/v), and keep this as the bulk (stock solution) at █°C ± █°C.

The manufacturing method proposed in the application did not require the addition of formalin to the virus suspension after desugaring procedure; however, the method was changed to one in which █% (v/v) formalin should be added as a stabilizer to the virus suspension, as described in the dossier submitted in █, 200█ (see “e. History of development of manufacturing process”).

c. Critical process steps, key intermediates, and process validation

The following 4 processes are defined as critical process steps.

Culture process: This is a process to propagate influenza virus. Changes with incubation time in HA titer and chicken red cell agglutination (CCA) titer were investigated. The results showed that incubation

conditions are validated.

 ultracentrifugation and ultracentrifugation: These processes are capable of removing process-derived impurities, thereby increasing the purity of the product. Validation data for the removal rate of the major impurities, ovalbumin and endotoxin, and the like has been provided, revealing that purification conditions for each process are validated.

Inactivation process: This is a critical process to inactivate the purified virus suspension prior to inactivation. Validation data for the first 3 lots of the bulk manufactured on a pilot scale has been provided, revealing that virus is inactivated within █ weeks when the protein concentration is █ to █ µg/mL.

Filtration process: This is a critical process to sterilize the stock solution.

d. Control of materials of human or animal origin

SPF embryonated eggs to be used in the production of the seed are collected from adult chickens in which 23 kinds of pathogens, specified in the WHO guideline (WHO TRS No.927, 2005) for inactivated influenza vaccines, are not detected. These adult chickens, from which embryonated eggs used for the manufacture of the bulk are to be collected, are bred appropriately, hygiene-controlled, and visually inspected daily, in conformity to the “Feeding hygiene control standard” based on the Domestic Animal Infectious Disease Control Law (Article 12-3), dated September 2004 and “Specific livestock infectious disease epidemic prevention guide for highly pathogenic avian influenza” published by the Ministry of Agriculture, Forestry and Fisheries, dated November 18, 2004. These chickens are also inoculated with vaccines against fowl pox, Newcastle disease, avian infectious bronchitis, infectious bursa, infectious laryngotracheitis, avian infectious coryza, Marek's disease, avian encephalomyelitis, and egg drop syndrome, and increased antibody titers to these viruses are to be identified in the chickens. Embryonated eggs are examined periodically for *salmonella* spp. and *Escherichia coli*.

e. History of development of the manufacturing process

Bulk lots used for non-clinical and clinical studies were manufactured without adding formalin after desugaring procedure; however, long-term test data failed to demonstrate adequate stability of either the bulk or the drug product [see “(1) Bulk, 6) Stability” and “(2) Drug Product, 4) Stability”]. Therefore, it was proposed after submission of the application that formalin be added at █% (v/v) as a stabilizer in the same manner as existing vaccines. The test included in the specifications was performed for 3 lots each of the bulks manufactured with and without formalin and 3 lots each of the drug products manufactured from their stock solutions. Consequently, there were no differences in quality among the lots, irrespective of the presence or absence of formalin, nor were there differences in actual measurements among the lots of the drug product used in clinical studies. Currently, pharmacology study

(for determining antibody titer) is ongoing to compare the drug products manufactured with and without formalin, and stability testing is ongoing as well for the bulk manufactured with formalin and the drug product manufactured from the bulk thus obtained. The details are shown in “(1) Bulk, 6) Stability” and “(2) Drug Product, 4) Stability.”

2) Characterization

The mock-up vaccine stock solution, which was manufactured using the NIBRG-14 strain according to the manufacturing method proposed in the application, was analyzed by electron microscopic observation, SDS-PAGE, sucrose density-gradient centrifugation, and high-performance liquid chromatography (HPLC). Also, base sequence analysis of the HA gene from RNA extracted from the bulk was conducted in comparison with the base sequence of the HA gene of the NIBRG-14 strain measured by the NIBSC. Consequently, the base sequences were all homologous and no mutations were detected. From the above characterization findings, it can be concluded that the bulk vaccine is composed of influenza virions.

3) Impurities

The following impurities derived from the manufacturing process are evaluated in terms of the removal rate: embryonated egg-derived ovalbumin, antibiotics used in the culture process, sodium citrate and purified sucrose used in the purification process, formaldehyde used in virus inactivation, and endotoxin. Since the purification process is consistent even after changing the manufacturing method without adding a stabilizer to that with the formalin stabilizer, the data obtained by the manufacturing method proposed in the application was used for the explanation of all impurities except ovalbumin and antibiotics.

The removal rate of ovalbumin was 99.76% to 99.86% by the [REDACTED] ultracentrifuge and 57.60% to 69.41% by the [REDACTED] ultracentrifuge, revealing that ovalbumin can be consistently removed. The maximum ovalbumin value actually measured for 5 bulk lots was 2.8 ng/mL and the maximum value following the dilution in the formulation process was 0.50 ng/mL; these values were much lower than “less than 5 µg/dose” recommended by the WHO (WHO TRS No.927, 2005). Thus, it was determined that there is no concern about ovalbumin in the light of manufacturing process-derived impurities. The levels of gentamicin sulfate and kanamycin sulfate contained in the bulk were shown to be below the detection limit. Sodium citrate and purified sucrose were removed efficiently by both the [REDACTED] ultracentrifuge and the ultrafiltration and by the ultrafiltration process, respectively. Also, because both sodium citrate and purified sucrose are used for foods, there would be no safety problems with using these substances. Endotoxin content is controlled as part of the specifications for the bulk. The present specification testing revealed that the endotoxin content of the stock solution slightly differed depending on the time of manufacture. However, the maximum level of residual endotoxin even in the diluted final bulk was only 9.3 EU/dose, substantially conforming to the acceptance criteria in the specifications for the bulk. Thus, endotoxin is not considered to pose safety problems.

4) Specifications and test methods

The following are included in the specifications for the bulk: sterility test, staining (Gram staining), inactivation test (for confirming the absence of virus proliferation after █ passages of subculture of virus in embryonated eggs), protein content, pyrogen test, HA content (by single radial immunodiffusion [SRD]), bacterial endotoxins test, pH, thimerosal content, and formaldehyde content.

Regarding the test for measuring HA content (by SRD), when SRD reagents [standard antigen and antiserum, see “5) Reference standard or standard substance”] are not available, the HA content is calculated by multiplying the protein content by the percent HA content. The percent HA content of the purified virus suspension prior to inactivation is determined by SDS-PAGE as an in-process control test.

The following are not included in the specifications for the bulk. Virus content (CCA titer), which is used to determine the content of active ingredients, is not defined as a specification because HA content (by SRD), included in the specifications for the present application, allows the determination of the content of active ingredients. The abnormal toxicity test, which is defined for subdividing of the product (as a final container) according to the Minimum Requirements for Biological Products of Influenza Vaccine, is not included in the specifications for the bulk but included in the specifications for the drug product in the present application. The test for determining leukocyte reduction in mice, which reflects the reaction of endotoxin and virus membrane component, is not defined, because the pyrogen test included in the specifications for the bulk is capable of examining both endotoxin and virus membrane component. The test for examining weight loss in mice is not included, because it is replaced by the abnormal toxicity test in guinea pigs having a higher sensitivity than mice, which is included in the specifications for the drug product. Osmolality ratio is not included in the specifications for the bulk because it is included in the specifications for the drug product.

5) Reference standard or standard substance

Reference standards used for specifications for the bulk included: standard influenza HA antigen (for SRD), reference anti-influenza HA antiserum, and standard albumin for protein assay, which are all supplied and change-controlled by the National Institute of Infectious Diseases. The storage condition of standard influenza HA antigen (for SRD) was changed from █°C ± █°C to █ ± █°C during development for lots used for the quality testing of the bulk manufactured according to the method proposed in the application, and from █°C ± █°C to █°C ± █°C for lots used for the quality testing of the bulk manufactured with the addition of formalin. The storage condition is █°C ± █°C for reference anti-influenza HA antiserum and █°C ± █°C for standard albumin for protein assay. No standard substance is used.

6) Stability

The following are the results of the tests performed for the stability of the bulk manufactured according to the method proposed in the application.

Long-term testing of the bulk manufactured without adding a stabilizer was conducted under the condition of $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and the following 8 parameters included in the specifications for the bulk were investigated: sterility, staining, protein content, HA content (by SRD), pH, inactivation, pyrogen, and formaldehyde content. A 27-month duration was planned for the testing, and the data was obtained every 3 months for the first 12 months. However, the HA content (by SRD) decreased with time and reached below the specified value (not less than $100 \mu\text{g HA/mL}$ by converting into $100 \mu\text{g/mL}$ protein content) at and after 9 months; therefore, the HA content test was terminated at 12 months. For all other tests except the HA content test (by SRD), the obtained results conformed to the acceptance criteria until 12 months. The accelerated testing was conducted at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 6 months; and the results showed that the HA content (by SRD) decreased from 1 month onward. From the above results, the expiration period of the bulk manufactured according to the method proposed in the application was determined to be 6 months from the manufacturing date.

Because adequate stability was not obtained for the bulk manufactured according to the method proposed in the application, as stated above, the stability testing of the NIBRG-14 strain bulk added with the formalin stabilizer was planned after the application and started in 2010 . The long-term testing of 18 months was planned, and the following were included in the specifications for the bulk, in addition to the aforementioned 8 parameters: thimerosal content, abnormal toxicity, bacterial endotoxins, characterization (electron microscopic observation to evaluate the higher-order structure, and the sucrose density-gradient fractionation), and state of formulation (to determine whether it is possible to formulate an appropriate drug product from the bulk stored for each storage period). The following were included in the specifications for a 6-month accelerated test scheduled: protein content, HA content (by SRD), and pH. For the separately manufactured bulk vaccine from A/Indo/05/2005 (H5N1) PR8-IBCDC-RG2 strain (hereinafter referred to as the Indonesian strain), the long-term testing was also started in 2010 , in order to determine protein content, HA content (by SRD), characterization, abnormal toxicity, and whether formulation is appropriate or not.

(2) Drug product

1) Product formulation and manufacturing method

Given that a large supply of the H5N1 Vaccine is required within a short period of time in the event of a pandemic, it is considered necessary to use an adjuvant which allows even a small amount of antigen (bulk) to exert sufficient effects. Therefore, aluminum hydroxide gel was selected as an adjuvant because its safety has been demonstrated from usage experience, such as in adsorbed hepatitis B vaccine and adsorbed diphtheria-purified pertussis-tetanus combined vaccine. Furthermore, since it is highly likely that people would be vaccinated on a population basis in the even of a pandemic, 2 doses, i.e., 1

mL and 10 mL drug products, were set and thimerosal, which has previously been used as a preservative of vaccine, was used.

The H5N1 Vaccine is an injectable suspension containing, per mL, 10 or 30 µg (on an HA content basis) of inactivated pandemic influenza virus and 0.3 mg (on an aluminum content basis) of aluminum hydroxide gel as an adjuvant. Other excipients included per mL are: 2.5 mg of sodium hydrogen phosphate hydrate, 0.4 mg of potassium dihydrogen phosphate, and not more than 8.1 mg of sodium chloride; and 10 µg of the thimerosal preservative was also included in 1 mL. A total of 4 types of drug products have been submitted for regulatory review. Those were 4 glass vials filled with 1 mL volume containing 10 µgHA/mL, 1mL volume containing 30 µgHA/mL, 10 mL volume containing 10 µgHA/mL, and 10 mL volume containing 30 µgHA/mL, respectively.

The method for manufacturing the drug product is as follows. Weigh the bulk based on the HA content of the bulk (determined by SRD, when standard influenza HA antigen [for SRD] is available, but determined by multiplying the protein content by the percent HA content previously obtained by SDS-PAGE, when standard influenza HA antigen is not available), dilute with PBS, add thimerosal solution and then aluminum hydroxide gel, as prepared below, gradually agitate at $\blacksquare^{\circ}\text{C} \pm \blacksquare^{\circ}\text{C}$ for \blacksquare hours, and obtain the final bulk. Aluminum hydroxide gel is prepared by mixing and agitating \blacksquare solution and \blacksquare solution, and the obtained gel is steam-sterilized under high pressure. The following are included in the specifications for the aluminum hydroxide gel: sterility, pH, and aluminum content.

Fill the final bulk into glass vials while agitating it to prepare the drug product.

The final bulk preparation process and the filling process are defined as the critical process steps.

2) Specifications and test methods

The following were included in the specifications for the drug product at the time of application: sterility, inactivation, protein content, potency (by SRD), pH, aluminum content, thimerosal content, formaldehyde content, abnormal toxicity, description, osmolality ratio, insoluble foreign matter, and extractable volume.

When SRD reagents are not available, the HA content is obtained by performing the test for determining the percent HA content, instead of the potency test (by SRD).

3) Reference standard and standard substance

The reference standard used for the specifications and test methods for the drug product was the same as that used for the specifications and test methods for the bulk.

4) Stability

The results of the tests for determining the stability of the drug product from the bulk manufactured according to the method proposed in the application are described below. To perform all the tests for determining the stability, the 1-mL and 10-mL drug products each containing 30 µgHA/mL and the 1-mL drug product containing 10 µgHA/mL were used. However, the 1-mL drug product containing 30 µgHA/mL was mainly used for the stability testing because the product was most likely to be used in clinical settings.

Long-term testing of the aforementioned drug product was conducted over a period of 15 months under the condition of $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$. For the 1-mL drug product containing 30 µgHA/mL, 14 parameters, including the protein content (precipitates) used for determining the amount of protein bound to the adjuvant, were assessed, in addition to 13 parameters included in the specifications. For the 10-mL drug product containing 30 µgHA/mL, the following 7 were included in the specifications for stability testing: sterility, protein content, potency (by SRD), pH, abnormal toxicity, description, and insoluble foreign matter. The following 6 were included in the specifications for the stability of the 1-mL drug product containing 10 µgHA/mL: protein content, protein content (precipitates), pyrogen, potency (by SRD), pH, and description.

Almost similar results were obtained for the 1-mL and 10-mL drug products each containing 30 µgHA/mL, indicating that stability does not differ depending on the volume. All tests except the potency test (by SRD) revealed that there were no chronological changes in stability for any of the drug products, while the potency test (by SRD) showed that the relative potency of all the drug products to reference standard were less than █ at 9 months. This result was lower than the acceptance criteria. For the drug product from the bulk manufactured by adding the formalin stabilizer, further tests are planned to be conducted, including the mouse antibody production test as potency indicator.

The same parameters as those for the long-term testing were included in those for the accelerated testing for up to 6 months at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The value obtained by SRD method was below the acceptance value from 1 month onward.

The photostability testing was conducted by exposing the product in a non-light-shielded or light-shielded (with aluminum foil) condition to not less than 1.2 million lux hours and an integrated near UV energy of not less than 200 watts per hour per square meter at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 11 days. No HA content was detected in the potency test (by SRD) of the non-light-shielded product, suggesting that HA antigen degradation where no antigen-antibody reaction occurred may have been induced by light. Additionally, the photostability testing was conducted for a vial packed individually in a paper box for commercial packaging under the aforementioned condition. The results revealed that the potency of the commercially packaged product was retained, as determined by the SRD method; thus, it can be

concluded that commercial packaging of the product prevents light exposure, even though the product is light-sensitive.

From the above results, the expiration period of the H5N1 Vaccine was determined to be 6 months when it is stored under light-shielded conditions at $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$. For the drug product from the bulk manufactured by adding a stabilizer, the long-term testing, accelerated testing, and photostability testing are ongoing, and the potential prolongation of the expiration period must await the testing results.

Outline of review by PMDA

Since the manufacturing method was changed from non-addition to addition of a stabilizer to the bulk shortly after submission of the application, many additional results of quality testing of the drug product were thereafter submitted. Also, because there are ongoing tests such as stability test, some items, including the specifications, for both the bulk and the drug product will be stated in the Review Report (2) following submission of the additional data.

(1) Seed lot system

PMDA asked the applicant to explain the handling of other virus strains than the NIBRG-14 strain and the time required for the establishment of the seed lot system, because the seed for the NIBRG-14 strain had been supplied without establishing the seed lot system from the National Institute of Infectious Diseases for the initial manufacturing. PMDA also recommended that the number of subcultures be minimum, because the WS for the NIBRG-14 strain was designated as corresponding to the ■th subculture of the original seed. The applicant responded as follows. For the separately manufactured vaccine from the Indonesian strain, the original seed supplied from the National Institute of Infectious Diseases was used to prepare the MS/WS for the manufacture of the bulk. Once SPF eggs are obtained, at least ■ days are required to prepare a single seed lot, and it takes further ■ weeks to test the seed. When the MS and the WS are prepared separately, double-time is required. For the NIBRG-14 strain, the seed prepared from the MS supplied from the National Institute of Infectious Diseases is to be used as the WS lot in place of using the MS/WS.

PMDA recognizes the possible need for starting the manufacture of pandemic vaccine shortly after the preparation of the seed (MS) in the event of an emergency, such as the onset of a pandemic; however, it proposed that a seed lot be prepared, given that the seed lot system should be established, in principle, in the less-urgent situation, i.e., in a pre-pandemic setting. The applicant responded as follows. The seed lot system is planned to be, in principle, established, although the MS may be used as the WS when the manufacture of pandemic vaccine is urgently required once a pandemic occurs. The applicant has so far requested the National Institute of Infectious Diseases to perform both the test for identifying the base sequence of the HA gene to characterize the seed and the HI test for identifying the antigenicity. In further, however, the applicant will lay out the framework to perform the seed control test in-house through the

technology transfer from the National Institute of Infectious Diseases.

PMDA requested the applicant to clearly identify the virus content, etc., at the time of thawing of the seed, because the stability of the NIBRG-14 strain seed over a period of █ years or more has not yet been demonstrated and the stability of the seed may vary depending on different strains. The applicant responded that determination of infectivity titers (in embryonated eggs) and determination of HA titers (HA reaction) would be conducted as in-process control tests when the MS is thawed to prepare a new WS and when the first batch of consecutively manufactured batches is used as the WS.

PMDA accepted the above response.

(2) Manufacturing process control of the bulk

Because the inactivation process and purification process, as explained below, had been defined before the process (desugering process) was required associated with changes in manufacturing from non-addition to addition of a stabilizer after submission of the application [See “*Summary of the submitted data*, (1) Bulk, 1) Manufacturing method, e. History of development of the manufacturing process”], the actual results not only from the manufacturing without adding a stabilizer according to the method proposed in the application, but also from the manufacturing on a commercial scale with the addition of a stabilizer were evaluated. The data on the separately manufactured bulk vaccine from the Indonesian strain, provided after the submission of the application, was also evaluated. The manufacture of the bulk from the Indonesian strain always included the addition of a stabilizer.

1) Inactivation process

The inactivation process, which is the only process capable of inactivating the influenza virus, is thought to be a critical process step involved in safety.

In response to the fact that analytical validation data of the inactivation test, defined as an in-process control test, was not provided, PMDA requested the applicant to explain the detection limit and specificity of the inactivation test, including the influence of the formalin present in the sample. The applicant conducted the additional inactivation test and gave the following explanation. A virus suspension from the WS (█ EID₅₀/0.2 mL) was diluted █ times and the diluted suspension was tested in the presence and absence of █% (v/v) formalin. The results showed that the same amount of residual virus was detected, irrespective of the presence or absence of formalin. The detection limit was obtained with about █ times diluted virus suspension and with █ to █ times diluted virus suspension in the presence and absence of █% (v/v) formalin, respectively. The sensitivity of the inactivation test, irrespective of the presence or absence of formalin, was equivalent to or higher than the theoretical value calculated from the infectivity titer of the virus sample in any diluted virus suspension. These results showed that the inactivation test is not interfered with in the presence of formalin in a sample used in the inactivation process, suggesting that the inactivation test allows the detection of residual infectious

viruses with an appropriate sensitivity. In the inactivation test, however, whether virus proliferation occurs after the \blacksquare th subculture of the virus depends on HA reaction. Thus, when the sample containing a high concentration of virus is used, HA activity, a virus component, may sometimes be detected in the \blacksquare th to \blacksquare th subcultures of viruses, even though virus infectivity has already been inactivated. In fact, when all lots manufactured from the Indonesian strain, apart from those manufactured from the NIBRG-14 strain used for the data acquisition for the application, were used at a protein concentration of \blacksquare $\mu\text{g/mL}$ for virus inactivation, the HA reaction was detected in up to \blacksquare th subculture of virus (Table 2). Thus, when samples containing infectious virus are used, the HA titer appears to increase in the \blacksquare th subculture of virus because of virus proliferation. This will be confirmed by the future validation of the specificity. PMDA understood the above explanation, but will discuss it in Review Report (2), along with the results of the specificity test to be thereafter submitted.

Table 1 shows the actual results of the inactivation process of all lots of the NIBRG-14 strain bulk. The concentration of formaldehyde was measured in the first 3 lots, and the results showed that the value was extremely lower in Lot \blacksquare than in the other lots. PMDA asked the cause of the extremely low value to the applicant. The applicant responded that the cause is still unclear. However, Lot \blacksquare was found to have an extremely high pyrogen activity in the pyrogen test, and the abnormal toxicity test of the lot also revealed that the guinea pig receiving it had a marked weight loss associated with a poor general condition; the testing was terminated and the animal was necropsied for the examination. Based on these findings, PMDA considers that safety concerns may arise when sufficient virus inactivation is not achieved due to a lowered concentration of formaldehyde, etc., even though infectious virus has no longer been detected.

Table 1: Inactivation Treatment Conditions and Inactivation Timing in the Manufacture of the Bulk from the NIBRG-14 Strain

Lot Number	Inactivation Treatment Condition							
	Protein Content Test ($\mu\text{g/mL}$)	Formaldehyde Content Test (w/v%)	HA Test (Times)	Inactivation Treatment Days (Day)	Inactivation Timing (W: Week)	Nonconforming Period	Temperature ($^{\circ}\text{C}$)	Sugar Concentration (%)
04-P-1								
04-P-2								
04-P-3								
05-P-1								
05-P-2								
06-PF-1F								
06-PF-2F								
06-PF-3F								
06-PF-4								
06-PF-5								
06-PF-6								
06-PF-7								
06-PF-8								
06-PF-9								

PMDA also asked the applicant to explain the reason why virus inactivation occurred at a different time, in view of the fact that inactivation was confirmed \blacksquare weeks after the initiation of inactivation treatment for Lot series 04, and for Lots \blacksquare and \blacksquare it was not confirmed after \blacksquare weeks but confirmed after \blacksquare

weeks. The applicant responded as follows. There were no great differences in inactivation conditions, such as HA titer, protein content, amount of added formalin, or other process operations, between Lot series 04 and 06. However, as compared to Lot series 06 for which about █ L of the pre-inactivation purified virus suspension was treated in a █-L container, about █ to █ L was treated in a █-L container for Lot series 04. Therefore, the difference in the timing of inactivation was considered to have been caused by external factors, such as the larger empty space in the container, although the precise reason is still unknown. Furthermore, for Lot series 04 and 05, the agitation was performed by shaking the container by hand after the addition of formalin; for Lot series 06, however, the validated agitation condition was used. Considering that the identical manufacturing scale and agitation condition should be employed in the actual manufacturing process, the aforementioned variation in the timing of inactivation is unlikely to occur in the future manufacturing process.

PMDA asked the applicant the reason why the test for confirming virus inactivation was performed after █ weeks and after █ weeks for █, although the manufacturing method section of the application document states that the inactivation test should be performed █ weeks after the initiation of inactivation treatment. The applicant explained that it was impossible to perform the testing for a certain period because of circumstances at the manufacturing site, and thus, there was no alternative to perform the inactivation test █ weeks or █ weeks after the initiation of inactivation treatment.

Furthermore, PMDA requested the applicant to submit information on inactivation treatment for the separately manufactured lots from the Indonesian strain. Of all 21 lots, only 3 were subjected to the inactivation test after █ weeks, as specified in the application document. For the remaining 18 lots, the inactivation test was performed after █ weeks (Table 2). Although the inactivation was ultimately confirmed for all the lots, the minimum duration required for the achievement of inactivation was not determined. PMDA asked the applicant to explain that 4 lots manufactured from the Indonesian strain █, for which virus inactivation was not confirmed █ weeks but confirmed █ weeks after the initiation of inactivation treatment or inactivation test did not meet the criteria by █ weeks and inactivation was confirmed for the first time at █ weeks. The applicant responded as follows. In the case of the NIBRG-14 strain, virus inactivation was confirmed at █ weeks in 6 of 8 lots and the simultaneously detected protein content in these lots were in the range of █ to █ µg/mL, while the protein content in the bulk manufactured from the Indonesian strain was █ µg/mL. In view of this, the applicant considers the timing of inactivation to vary depending on the protein content.

Table 2: Inactivation Treatment Conditions and Inactivation Test Data (Confirmation Time of Inactivation) in the Manufacturing of the Bulk from the Indonesian Strain

Lot Number	Protein Concentration (µg/mL)	Confirmation of Inactivation After Adding Formalin (W: Week)	HA Titer (Geometric Mean)			Determination		
			E1	E2	E3	E1	E2	E3
06-PF-10								
06-PF-11								
06-PF-12								
06-PF-13								
06-PF-14								
06-PF-15								
06-PF-16								
06-PF-17								
06-PF-18								
06-PF-19								
06-PF-20								
06-PF-21								
06-PF-22								
06-PF-23								
06-PF-24								
06-PF-25								
06-PF-26								
06-PF-27								
06-PF-28								
06-PF-29								
06-PF-30								

The above findings indicate that the conditions of the inactivation process may affect the safety of the bulk. Thus, PMDA requested the applicant to manufacture the bulk in conformity to the specified inactivation conditions, including the following: (a) that additional validation for the inactivation process should be conducted on an actual manufacturing level, (b) that measurement of both the formaldehyde concentration and the protein content at the time of inactivation treatment should be included in the specifications for in-process control tests, and (c) that the inactivation test should be mandatory 1 weeks after the initiation of inactivation treatment. The applicant responded that the inactivation test would be performed in an appropriate manner by adopting the above conditions. PMDA considers it mandatory to show the results of further inactivation validation to be conducted at the time of actual manufacture of the bulk.

2) Purification process

PMDA evaluated the validity of the purification process in terms of the purification rate and the percent recovery.

Because the methods for measuring the active ingredient differ between the intermediates and the bulk, an increased purity of the active ingredient is of limited value in evaluating the purification rate. Therefore, the purification process was evaluated as follows, based on the impurity content obtained by using a highly quantitative testing approach through the processes.

PMDA asked the applicant to explain the status of removal of impurities. The applicant submitted additional data (Table 3) concerning the removal of ovalbumin in actual 5 lots from the bulk manufactured with the addition of a stabilizer, along with the validation data obtained on a pilot-scale manufacturing level, as presented in the summary of the application dossier. It has been shown that impurities were removed by not less than 99% using the [redacted] ultracentrifuge, the remaining impurities were removed by not less than about 60% using the [redacted] ultracentrifuge, and even more remaining impurities were removed by not less than about 90% in the subsequent [redacted] process. It was therefore determined that the residual rate was not more than about 0.01% by the time of the final filtration process through constant and effective removal processes of impurities. PMDA accepted the above response.

Table 3: Residual Content of Ovalbumin

Lot Number	Attribute	Incubation/ Concentration Process	Purification Process		Final Filtration Process
		Concentrated Virus Suspension	[redacted] Solution	[redacted] Solution	Stock Solution
06-PF-1	Ovalbumin Concentration	[redacted]	[redacted]	[redacted]	[redacted]
	Removal Rate from Previous Process	[redacted]	[redacted]	[redacted]	[redacted]
	Residual Rate	[redacted]	[redacted]	[redacted]	[redacted]
06-PF-2	Ovalbumin Concentration	[redacted]	[redacted]	[redacted]	[redacted]
	Removal Rate from Previous Process	[redacted]	[redacted]	[redacted]	[redacted]
	Residual Rate	[redacted]	[redacted]	[redacted]	[redacted]
06-PF-3	Ovalbumin Concentration	[redacted]	[redacted]	[redacted]	[redacted]
	Removal Rate from Previous Process	[redacted]	[redacted]	[redacted]	[redacted]
	Residual Rate	[redacted]	[redacted]	[redacted]	[redacted]
06-PF-4	Ovalbumin Concentration	[redacted]	[redacted]	[redacted]	[redacted]
	Removal Rate from Previous Process	[redacted]	[redacted]	[redacted]	[redacted]
	Residual Rate	[redacted]	[redacted]	[redacted]	[redacted]
06-PF-5	Ovalbumin Concentration	[redacted]	[redacted]	[redacted]	[redacted]
	Removal Rate from Previous Process	[redacted]	[redacted]	[redacted]	[redacted]
	Residual Rate	[redacted]	[redacted]	[redacted]	[redacted]

PMDA then asked the applicant to explain the percent recovery, and made the following comments based on the information shown in response. The results of actual 14 lots manufactured so far from the NIBRG-14 strain revealed that the protein content of the bulk obtained per egg was [redacted] ± [redacted] µg/egg, irrespective of the manufacturing scale and the presence or absence of the stabilizer. This indicates that the percent recovery of protein is stable. The percent HA content was [redacted] ± [redacted]%, and thus, the HA content of the stock solution derived from 1 egg was about [redacted] µgHA for the NIBRG-14 strain and slightly smaller than about [redacted] doses would be obtained from 1 egg when the clinical dose is defined as 15

µgHA/dose. In the light of the fact that a single manufacturing lot allows the treatment of ■ eggs, it would be possible to manufacture the bulk corresponding to about ■ dose per lot. On the other hand, the results of actual 21 lots that were separately manufactured from the Indonesian strain revealed that the HA content of the stock solution derived from 1 egg was about ■ µgHA, corresponding to slightly smaller than ■ doses. From these results, PMDA determined that the active ingredient could be recovered in a stable manner from the identical strain through the proposed manufacturing process, while PMDA considers it necessary to allow for propagation of the strain to be used, etc., in the embryonated eggs in establishing the manufacturing plan.

(3) Characterization of the bulk

PMDA considers it necessary to determine whether or not influenza HA vaccine of the H5N1 subtype produced by the reverse genetics approach retains the whole-virus structure with a lower antigenicity than seasonal influenza HA vaccine (*Phil. Trans. R. Soc. Lond.*, 2001; 356: 1953-1960) and a higher antigenicity than HA vaccine (*Virus Res.*, 2004; 103: 163-171, *Lancet*, 2003; 362: 1959-1966). Because the HPLC analysis revealed that the peak of the virions was outside the range of the molecular weight for the column, PMDA asked the applicant to explain about impurities detected by the analysis. The applicant described that when influenza HA vaccine was added, 2 eluted peaks of virions and HA existed at different elution time (just less than 1 minute). Focusing on the fact that the two peaks were closely located, it is unlikely that the HPLC analysis has enough ability to detect incomplete degradation of virions and slight breakup of virion. Therefore, PMDA considers it necessary to conduct a comprehensive evaluation of bulk characterization, including the results obtained from other methods, such as sucrose density-gradient centrifugation and electron microscopy, in detecting degradation of virions.

Whole virus influenza vaccine is known to have a tendency for higher stimulation etc. in animal tests (*Japan J. Med. Sci. Biol.*, 1975; 28: 37-52); therefore, PMDA requested the applicant, if available, to show the relevant data. The applicant explained as follows. In the abnormal toxicity test of the bulk lots ■ to ■ manufactured by the proposed method without adding a stabilizer, guinea pigs were vaccinated with 5 mL of vaccine containing ■ µg/mL of protein. Consequently, all of the lots were found to induce weight loss in the animals. Of 3 animals vaccinated with ■, 1 died and of another 3 animals vaccinated with ■, 1 had a marked weight loss and a worsening of general condition. Thus, the test was terminated, and these 2 animals were necropsied for the examination. However, it was unclear whether the death and pathogenesis in these animals were causally related to the inoculated material. Thus, further testing was conducted for these bulk lots 9 months after the manufacture; however, neither deaths nor marked weight loss was observed. For the simultaneously manufactured drug product from the same bulk, the abnormal toxicity test revealed no evidence of abnormalities.

It was impossible to clearly identify their causality to bulk inoculation, and thus, the cause of weight loss

and death is unknown. PMDA requested the applicant to further conduct the abnormal toxicity test using the bulk obtained shortly after the manufacture. The applicant explained the testing results as follows. For 3 lots, [REDACTED], of the bulk obtained shortly after the manufacture with the addition of a stabilizer, the following test samples were prepared for the abnormal toxicity test: (a) bulk containing [REDACTED] $\mu\text{g/mL}$ of protein (equivalent to or higher than the concentration of the final bulk), (b) bulk containing [REDACTED] $\mu\text{g/mL}$ of protein, and (c) drug product manufactured from the relevant bulk (containing [REDACTED] $\mu\text{g/mL}$ of protein). No abnormalities were observed in guinea pigs treated with the bulk containing [REDACTED] $\mu\text{g/mL}$; that is, the animals had gained body weight on Day 7 (last measurement) as compared with that before inoculation. Weight loss was less in a dose-dependent manner in the animals treated with the [REDACTED] $\mu\text{g/mL}$ bulk relative to that in the animals treated with the [REDACTED] $\mu\text{g/mL}$ bulk. Furthermore, although the amount of virus in the bulk containing [REDACTED] $\mu\text{g/mL}$ of protein was the same as that in the drug product manufactured from the relevant bulk, the animals showed weight loss but recovered earlier in the drug product group than in the bulk group. This suggests that the aluminum hydroxide gel adjuvant, used for the drug product, may have attenuated the adverse effect on body weight in the guinea pig model.

As stated in “*Outline of the Review by PMDA*, (2) Manufacturing process control of the bulk, 1) Inactivation process,” PMDA considers that the abnormalities observed for Lot [REDACTED] manufactured without adding a stabilizer in the early phase of development may have been attributed to insufficient inactivation control, such as inconsistent concentration of formaldehyde, and that the addition of the formalin stabilizer may have attenuated the stimulation to guinea pigs while inducing virus inactivation to the steady state. Considering that the drug product showed a decrease in weight loss, the stimulation may be attributable to a virus component to be adsorbed onto the adjuvant. In the pyrogen test, the bulk manufactured without adding the formalin stabilizer (according to the method proposed in the application) did not meet the acceptance criteria, although 2 of 3 lots met the criteria when the testing was repeated in heated samples. Furthermore, because all lots of the bulk manufactured with the addition of formalin met the criteria even in non-heated samples, PMDA considers that the addition of the formalin stabilizer would improve vaccine safety. For the 3 lots manufactured from the Indonesian strain, both the pyrogen test and the abnormal toxicity test were performed shortly after the manufacture of the bulk with the addition of the stabilizer; and no abnormalities were observed in either test.

Furthermore, the applicant made the comments. Safety testing of the H5N1 Vaccine was also conducted using leukocyte reduction and weight loss as indicators in mice. The results met the criteria obtained from the testing for previously approved whole influenza virus vaccine (without adjuvant) and posed no safety problems.

PMDA accepted the applicant’s explanation, because no problems were found in the bulk manufactured with the addition of the stabilizer and the toxicity attenuation was found in the abnormal toxicity test of the drug product. However, PMDA considers it necessary to conduct careful investigation by performing

the abnormal toxicity test, pyrogen test, etc. for the future manufacture of the drug product from a new strain, because toxicity profile may vary with different strains.

(4) Manufacturing process control of the drug product

Only 3 parameters (sterility, pH, and aluminum content) were included in the specifications for aluminum hydroxide gel at the time of application. PMDA asked the applicant whether the above parameters are enough to ensure the efficacy and safety of the drug product, because aluminum hydroxide gel is added as an adjuvant. The applicant responded that both the adsorption test and the abnormal toxicity test would be added and that data on virion size would be acquired as quickly as possible to determine whether it is possible to include virion size in the specifications. PMDA accepted the above response.

In the light of the fact that the H5N1 Vaccine is a suspension, agitation conditions at the time of filling, etc., homogeneity of the fill volume of active ingredient, adjuvant adsorption, and the like are considered important; however, the validation was conducted only for the adsorption rate of antigen protein to the adjuvant and the fill volume. PMDA asked the applicant to explain about the validation of homogeneity of the fill volume. The applicant responded that it is scheduled to determine the fill volume and content homogeneity on an actual manufacturing scale for a mock-up vaccine prepared by adding aluminum hydroxide gel alone and on a pilot scale level for an actual bulk solution. PMDA is to review the results to be obtained in the future.

(5) Analytical validation of the test methods

A number of tests have been added to the specifications and in-process control tests for both the bulk and the drug product, and analytical validation for these tests was conducted after application; however, such data presented for the application was unsatisfactory. Thus, PMDA asked the applicant as to whether to conduct analytical validation for the added tests again. The applicant responded that reinvestigation of a part of the results from about 30 tests is further scheduled. As the test results are being submitted as needed, PMDA is to review such data.

3. Non-clinical data

(i) Summary of pharmacology studies

Summary of the submitted data

(1) Primary pharmacodynamics

1) Antibody titer determination

A test solution containing whole inactivated influenza virus at a concentration of 30 µg HA/mL was prepared, and to this test solution, 0.3 mg/mL (on an aluminum content basis) of aluminum hydroxide gel was added to prepare another test solution containing the aluminum adjuvant. Both the test solutions

were diluted 1, 5, 25, and 125 times, and 100 μ L of the diluted solution (3 μ g, 0.6 μ g, 0.12 μ g, or 0.024 μ g as HA antigen) per animal was injected twice at a 3-week interval between the two doses intramuscularly into the femur or subcutaneously into the back of female BALB/c mice aged 5 weeks (10 animals per group). Also 100 μ L of a solution containing 0.03 mg of aluminum hydroxide gel and 100 μ L of physiological saline were used as the control solutions and administered similarly to the animals. Exsanguination was performed 2 weeks after the second administration, and the serum HI antibody and neutralizing antibody titers were measured.

In both the cases of intramuscular and subcutaneous injection, the mean HI antibody titer (geometric mean) and neutralizing antibody titer (geometric mean) increased basically in a dose-dependent manner within the dose range of 0.024 to 3 μ g of HA antigen. The geometric mean of the titers were higher in the animals vaccinated with the test solutions containing aluminum hydroxide gel than in those vaccinated with the test solutions not containing aluminum adjuvant for any dose of HA antigen. Higher HI antibody and neutralizing antibody titers were obtained with intramuscular injection than with subcutaneous injection.

(2) Summary of the results of pharmacology safety studies

1) Effects on the central nervous system

To male Crl:CD (SD) rats aged 5 weeks (6 animals in a group), 0.25 mL/kg or 0.5 mL/kg of the H5N1 Vaccine, corresponding to about 25 times or 50 times the clinical dose, was injected subcutaneously into the back as a single dose. No effects of the H5N1 Vaccine on the general condition or behavior of the rats were observed at either dose.

2) Effects on the cardiovascular and respiratory systems (telemetry)

To male beagle dogs aged 9 to 10 months (4 animals in a group), 0.25 mL/kg or 0.5 mL/kg of the H5N1 Vaccine, corresponding to about 25 times or 50 time the clinical dose, was injected subcutaneously into the back twice, with a 2-week interval between the doses. The heart rate, blood pressure (systolic, diastolic and mean blood pressure), PR interval, QRS width, and the QT interval and QTc were evaluated as the cardiovascular parameters, and the respiratory rate and arterial blood gas analysis parameters (pO₂, pCO₂, pH, and arterial hemoglobin O₂ saturation [%]) were evaluated as the respiratory parameters.

• Effects on the cardiovascular system

Transient shortening of the QT interval was observed after the first administration of 0.25 mL/kg of the H5N1 Vaccine, and a transient increase of the heart rate, narrowing of the QRS width, and shortening of the QT interval were observed after the second administration of 0.25 mL/kg. However, since no effects on the QTc were observed and no similar changes were observed after administration of 0.5 mL/kg of the H5N1 Vaccine, the above-mentioned changes were not considered to be caused by the vaccine itself.

- **Effects on the respiratory system**

Although sporadic changes of the blood gas parameters (pO₂, pCO₂, pH, and arterial hemoglobin O₂ saturation [%]) were noted after administration of both 0.25 mL/kg and 0.5 mL/kg of the H5N1 Vaccine, these changes were within the accepted physiologic changes for normal animals and no tendency towards dose dependence was observed. Therefore, the changes in these blood gas parameters were not considered to be caused by the H5N1 Vaccine itself. No effects were observed on the general condition or behavior of the animals at either dose.

From these findings, it was concluded that the H5N1 Vaccine has no effects on the central nervous system, cardiovascular system, or the respiratory system.

Outline of review by PMDA

Protective effect of H5N1 Vaccine against infection

PMDA concluded that there were no major problems with the results of the antibody titer determination performed to investigate the immunogenicity of the H5N1 Vaccine. However, because the results yield no direct evidence in support of the effect of the H5N1 Vaccine, PMDA asked the applicant to show results of a challenge test of the H5N1 Vaccine or analogous vaccine to investigate the potential protective effect of the H5N1 Vaccine against infection.

The applicant responded as follows.

Since no challenge test has been performed using the H5N1 Vaccine, there is no direct evidence in support of the protective effect of the H5N1 Vaccine against infection. However, Ninomiya et al. of the National Institute of Infectious Diseases performed mouse challenge tests using a vaccine containing whole inactivated influenza virus (attenuated strain NIBRG-14, derived from Clade* 1 virus), as with the H5N1 Vaccine, as the active ingredient, and the aluminum adjuvant. Administration of this vaccine induced increases in both the serum HI antibody and neutralizing antibody titers. In addition, in a test of transnasal infection with a virulent strain (A/Viet Nam/1203/04), 90% of the non-vaccinated mice died, while all (100%) of the vaccinated mice survived, showing the protective effect of this vaccine against the infection (“Results of mouse immune challenge test using NIBRG-14 vaccine,” December 25, 2006, internal document of Saikin Seizai Kyoukai).

Furthermore, Ninomiya et al. reported that administration of a vaccine derived from the above-mentioned NIBRG-14 strain (Clade 1) also induced the production of neutralizing antibodies against the Clade 2 virus, which has a different antigenicity profile from Clade 1, and showed protective effect against infection with the virulent Clade 2 strain (the shared research report entitled “Study on efficacy of a

* The origin of the term Clade is “branch” in Greek, which means a branch of a dendrogram in taxonomy. In the case of the H5N1 viruses, it refers to a group of viruses classified based on the homology of gene sequences in the dendrogram. H5N1 strains which have been isolated since 2003 are classified into Clade 1 and Clade 2. The Clade 1 and Clade 2 show considerable differences in the antigenicity. The Clade 2 viruses are further classified into subClade viruses 1, 2, and 3.

vaccine for pandemic influenza virus in mice” [<http://mhlw-grants.niph.go.jp/niph/search/NIST00.do>, 54th meeting of the Japanese Society for Virology, 2006; Presentation Summary No.3B26] of Regulatory Science of Pharmaceuticals and Medical Devices “Research on the efficacy and safety of a vaccine for pandemic influenza” supported by a Health and Labour Sciences Research Grant in 2006).

From the data described above, it can be expected that the H5N1 Vaccine would have a protective effect against infection with the virulent strain Clade 1 (A/Viet Nam/JP1203/04) and also a cross-protective effect against infection with the Clade 2 strain of virus which possesses a different antigenicity profile.

PMDA made the following comments. It is considered difficult to obtain clinical study data demonstrating the protective effect of the H5N1 Vaccine against infection with pandemic influenza viruses at the present time, which has not yet occurred, and it is impossible to determine whether all the results obtained in the mouse experimental model would also be applicable to humans. Nevertheless, it is of great value in terms of the clinical efficacy of the H5N1 Vaccine to accept the aforementioned data showing that the vaccine containing whole inactivated influenza virus (NIBRG-14 strain) as the active ingredient and aluminum adjuvant, as with the H5N1 Vaccine, has the protective effect and also cross-protective effect against infection. The applicant submitted the quotation from the shared research report of the Health and Labour Scientific Research as the response to PMDA, stating that “even though the mouse blood antibody titers were below the detection limit or not significantly elevated after administration of the vaccine, the vaccine can protect the mice against the cross-antigenic viral challenge.” Similar results were also shown in a report of a viral challenge test in ferrets (*Nat. Rev. Microbiol.*, 2006; 4: 565-566). These data suggest that post-vaccination immune responses other than specific antibody production and cellular immunity may be involved in the protective effect of the product against the infection.

PMDA concluded that there were no particular problems with the submitted results of the pharmacology safety studies.

(ii) Summary of pharmacokinetic studies

No pharmacokinetic studies were conducted.

(iii) Summary of toxicology studies

Summary of the submitted data

(1) Single-dose toxicity

Single-dose toxicity of the H5N1 Vaccine was examined following subcutaneous administration in rats and dogs.

Rats were given the H5N1 Vaccine at doses of 2 and 10 mL/kg, corresponding to 200 and 1000 times the

maximum clinical dose (30 µg HA/mL drug product was used, which contains 15 µg HA protein/dose), respectively (the same applies hereinafter). No deaths or abnormalities in the general condition were observed at either dose. At necropsy, subcutaneous induration at the injection site was observed in the 2 dose groups, presumably caused by the aluminum hydroxide gel. No abnormalities were detected in other organs. From the above results, the approximate lethal dose in rats was determined to be ≥ 10 mL/kg.

Dogs were given the H5N1 Vaccine at doses of 1 and 5 mL/kg, corresponding to 100 and 500 times the maximum clinical dose, respectively. No deaths were encountered at either dose. At necropsy, subcutaneous induration at the injection site was observed in the 2 dose groups, presumably caused by the aluminum hydroxide gel. No abnormalities were detected in other organs. From the above results, the approximate lethal dose in dogs was determined to be ≥ 5 mL/kg

(2) Repeat-dose toxicity

Repeat-dose toxicity of the H5N1 Vaccine was examined in a 4-week subcutaneous administration study in rats, in which the H5N1 Vaccine was administered at doses of 0.25 and 0.5 mL/kg, corresponding to 25 and 50 times the maximum clinical dose, respectively, 5 times at intervals of 1 week. At both doses and in both sexes, subcutaneous induration was noted macroscopically and granulomatous inflammation was observed histopathologically at the injection site, which was considered to represent an immunological reaction to the H5N1 Vaccine. Furthermore, hyperplasia of the germinal centers of the axillary lymph nodes was noted at 0.5 mL/kg in both sexes, which was considered to represent a secondary reaction to the granulomatous lesions at the injection site. In blood biochemistry, a low A/G ratio and high β globulin level, as also an elevated γ -globulin level, were observed in females in the 0.5 mL/kg group as compared with the values in the control group. The same tendency was also seen in females in the 0.25 mL/kg group. These changes were considered to be related to stimulation of immunoglobulin production by the H5N1 Vaccine. There were no other abnormalities, including in relation to the general condition, attributable to administration of the H5N1 Vaccine. The no-observed-adverse-effect level (NOAEL) was estimated to be > 0.5 mL/kg in both sexes under the experimental conditions of this study.

(3) Reproductive and developmental toxicity

The effect of the H5N1 Vaccine on fertility and early embryonic development to implantation was evaluated in a 4-week subcutaneous administration study in rats, by histopathological examination, etc., of the reproductive organs of animals of both sexes. No abnormalities were detected; therefore, the H5N1 Vaccine was considered to have no adverse effects on the reproductive function under the conditions employed in this study.

In a study on the effect of the H5N1 Vaccine on embryo-fetal development, doses of 0.25 and 0.5 mL/kg,

corresponding to 25 and 50 times the maximum clinical dose, respectively, were administered subcutaneously 3 times, on Days 7, 12, and 17 of gestation. No toxic effects of the H5N1 Vaccine were noted. From the above results, NOAEL was estimated to be > 0.5 mL/kg for general toxicological effects in relation to both reproductive function in dams and embryo-fetal development under the conditions employed in this study.

A study on the effect of the H5N1 Vaccine on pre- and postnatal development and maternal function is underway.

(4) Local irritation

The potential of the H5N1 Vaccine to cause local irritation at the sites of intramuscular and subcutaneous administration was examined in rabbits after administration of a single dose and two repeat doses.

In the single-intramuscular administration study, the H5N1 Vaccine was injected into the lateral great muscle of one side and physiological saline was injected into the corresponding muscle on the other side. KIB-PI (preparation not containing aluminum hydroxide gel) and adsorbed diphtheria-purified pertussis-tetanus combined vaccine (DPT vaccine) were injected into the corresponding sites in the reference control group, and 0.75% acetic acid and 6% acetic acid were injected on either side in the positive control group. The dosing volume in all cases was 0.5 mL. The animals were necropsied and macroscopic and histopathological examinations were performed 2 and 7 days after the administrations. In the repeat-administration study, the H5N1 Vaccine was injected into the lateral great muscle of one side on Day 1 as the first dose and into the corresponding muscle of the other side on Day 14 as the second dose. KIB-PI and DPT vaccine were injected into the corresponding sites in the reference control group. The dosing volume in all cases was 0.5 mL. The animals were necropsied and macroscopic and histopathological examinations were conducted 2 and 7 days after the second administration (Days 16 and 21 after the first administration). Macroscopic examination after single administration of the H5N1 Vaccine revealed very mild signs of irritation on Day 2, which disappeared by Day 7. In the repeat-administration study, evidence of very mild irritation was observed at the second injection site 2 and 7 days after the administration both for the H5N1 Vaccine and the DPT vaccine. Histopathological examination after single administration of the H5N1 Vaccine revealed retention of the administered substance and minimal or mild inflammatory cell infiltration, degeneration of muscle fibers, and hemorrhage on Day 2. On Day 7, only minimal inflammatory cell infiltration was noted. On histopathological examination after repeated administration of the H5N1 Vaccine, inflammatory cell infiltration and degeneration of muscle fibers were observed at the first injection site, and retention of the administered product and hemorrhage were observed at the second injection site. From the above results, the potential of the H5N1 Vaccine to cause local irritation at the site of intramuscular injection was considered to be equal to that of the DPT vaccine.

In the single-subcutaneous administration study, the H5N1 Vaccine was injected into the abdominal wall on one side and physiological saline on the other side. KIB-PI and DPT vaccine were injected into the corresponding sites in the reference control group. The dosing volume in all cases was 0.5 mL. The animals were necropsied 2 and 7 days after the administrations. In the repeat-subcutaneous administration study, the H5N1 Vaccine was injected into the abdominal wall on one side as the first dose and into the abdominal wall on the other side 14 days later as the second dose. KIB-PI and the DPT vaccine were injected into the corresponding sites in the reference control group. The dosing volume in all cases was 0.5 mL. Necropsy was conducted 2 and 7 days after the second administration (Days 16 and 21 after the first administration). Histopathological examination revealed slightly severe inflammatory cell infiltration on Day 7 after the single administration of the H5N1 Vaccine. At the site of the second injection for repeat administration, relatively severe inflammatory cell infiltration was observed on Day 2 after the administration for the DPT vaccine, while only minimal inflammatory cell infiltration was observed at the site of injection of the H5N1 Vaccine. On Day 7 after the administration, only minimal inflammatory cell infiltration was noted in all groups. From the above results, the potential for the H5N1 Vaccine to produce local irritation at the site of subcutaneous administration was considered to be slightly less than that of the DPT vaccine.

Outline of review by PMDA

Through adequate pre-application guidance and advice obtained from PMDA, toxicological issues had already been resolved by the time of the application, and therefore, no issues were raised by PMDA at the time of the review.

4. Clinical data

Summary of the submitted data

The results of 1 Japanese phase I study and 1 Japanese phase II/III study were submitted as efficacy and safety evaluation data. These studies and their major efficacy data are summarized in Table 4.

Table 4: Summary of clinical studies and their efficacy data (Study KIB-PIA01, Study KIB-PIA02)

Area	Study Number	Phase	Subjects	Dosage and administration	Number of subjects	Primary endpoint	Major findings
Japan	KIB-PIA01	I	Healthy male adults (20-40 years of age)	1.7 µg/5 µg/15 µg 2 injections at an interval of 21 (± 2) days, subcutaneous or intramuscular	20/group	Safety/ immunogenicity	541 adverse events in 113 of 120 subjects (94.2%), 279 adverse reactions in 81 of 120 subjects (67.5%). Changes in neutralizing antibody titer (geometric mean) after subcutaneous injection 1.7 µg group, 5.7→10.7→12.7 5 µg group, 5.6→17.9→29.9 15 µg group, 6.0→27.8→41.5
	KIB-PIA02	II/III	Healthy adults (20-64 years of age)	5 µg/15 µg 2 injections at an interval of 21 (± 7) days, intramuscular	5 µg group, 150 15 µg group, 150	Immunogenicity	Seroconversion rate (neutralizing antibody titer) 5 µg group, 65.1% 15 µg group, 80.5%

(1) Japanese phase I study (Study Number, KIB-PIA01; Attachment, 5.3.5.1-1; Publication, None; Study Period, ■■■ 20■■■ to ■■■ 20■■■)

An open-label study was conducted at a single study site in Japan to evaluate the safety and immunogenicity of the H5N1 Vaccine in healthy male adults.

The investigational vaccine (1.7, 5, or 15 µg in terms of HA content) was to be administered subcutaneously or intramuscularly in the upper arm twice at an interval of 21 ± 2 days.

All 120 vaccinated subjects (20 per group) were included in the safety analysis. Excluding 2 cases of discontinuation of the H5N1 Vaccine for the second administration (1 subject receiving 5 µg subcutaneously and 1 subject receiving 15 µg intramuscularly) and 2 cases of one-week delay in the second administration (1 subject receiving 15 µg subcutaneously and 1 subject receiving 15 µg intramuscularly), the remaining 116 subjects were included in the Per Protocol Set (PPS). HI antibody titers (using equine and chicken erythrocytes) and neutralizing antibody titer were assayed in the PPS for calculation of the geometric mean and geometric standard deviation (Table 5).

Table 5: Changes in HI antibody titers (Study KIB-PIA01, PPS)

Dose group	Number of subjects included	Subcutaneous						Number of subjects included	Intramuscular					
		HI antibody titer (equine erythrocytes)							HI antibody titer (equine erythrocytes)					
		Prior to first administration		Prior to second administration		Post-study investigation			Prior to first administration		Prior to second administration		Post-study investigation	
Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation			
1.7 µg	20	5.0	1.00	5.5	1.59	6.2	1.74	20	5.0	1.00	7.3	2.07	8.4	2.31
5 µg	19	5.0	1.00	7.2	2.25	8.3	2.21	20	5.0	1.00	7.8	2.41	10.0	2.53
15 µg	19	5.0	1.00	10.0	2.88	12.9	2.83	18	5.4	1.39	10.4	2.70	15.9	2.28
Dose group	Number of subjects included	HI antibody titer (chicken erythrocytes)						Number of subjects included	HI antibody titer (chicken erythrocytes)					
		Prior to first administration		Prior to second administration		Post-study investigation			Prior to first administration		Prior to second administration		Post-study investigation	
		Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation		Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation
1.7 µg	20	5.0	1.00	5.0	1.00	5.0	1.00	20	5.0	1.00	6.2	1.58	6.8	1.85
5 µg	19	5.0	1.00	6.2	1.68	6.7	1.87	20	5.0	1.00	6.2	1.58	6.8	1.77
15 µg	19	5.0	1.00	6.9	1.87	7.5	2.10	18	5.0	1.00	7.9	2.12	10.0	2.12
Dose group	Number of subjects included	Neutralizing antibody titer						Number of subjects included	Neutralizing antibody titer					
		Prior to first administration		Prior to second administration		Post-study investigation			Prior to first administration		Prior to second administration		Post-study investigation	
		Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation		Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation	Geometric mean	Geometric standard deviation
1.7 µg	20	5.7	1.33	10.7	1.24	12.7	1.59	20	6.2	1.49	18.0	2.62	23.0	2.84
5 µg	19	5.6	1.30	17.9	3.12	29.9	3.27	20	5.4	1.24	18.0	2.96	47.6	3.21
15 µg	19	6.0	1.48	27.8	3.42	41.5	2.99	18	5.4	1.25	38.5	3.32	58.8	2.06

A total of 541 adverse events were reported by 113 of 120 subjects (94.2%). Two hundred seventy-nine adverse events for which a causal relationship to the H5N1 Vaccine could not be denied (classified as adverse reactions) were reported by 81 of 120 subjects (67.5%): 60 reactions in 16 of 20 subjects (80.0%) in the subcutaneous 1.7 µg group, 8 reactions in 7 of 20 subjects (35.0%) in the intramuscular 1.7 µg group, 72 reactions in 18 of 20 subjects (90.0%) in the subcutaneous 5 µg group, 11 reactions in 8 of 20 subjects (40.0%) in the intramuscular 5 µg group, 110 reactions in 20 of 20 subjects (100.0%) in the subcutaneous 15 µg group, and 18 reactions in 12 of 20 subjects (60.0%) in the intramuscular 15 µg group. Adverse reactions reported by 10% or more of the subjects in each dose group are summarized in Table 6.

**Table 6: Adverse reactions reported by 10% or more of the subjects in each dose group
(Study KIB-PIA01, safety analysis set)**

	Subcutaneous						Intramuscular					
	1.7 µg group		5 µg group		15 µg group		1.7 µg group		5 µg group		15 µg group	
Number of subjects included	20		20		20		20		20		20	
System Organ Class/Preferred Term	Number of Subjects	Incidence	Number of Subjects	Incidence	Number of Subjects	Incidence	Number of Subjects	Incidence	Number of Subjects	Incidence	Number of Subjects	Incidence
Local reactions												
Injection site erythema	13	65.0%	14	70.0%	19	95.0%	0	0.0%	2	10.0%	0	0.0%
Injection site induration	7	35.0%	6	30.0%	11	55.0%	0	0.0%	0	0.0%	0	0.0%
Injection site pruritus	5	25.0%	3	15.0%	5	25.0%	0	0.0%	0	0.0%	0	0.0%
Injection site warmth	3	15.0%	3	15.0%	11	55.0%	0	0.0%	0	0.0%	0	0.0%
Injection site swelling	4	20.0%	5	25.0%	16	80.0%	0	0.0%	0	0.0%	0	0.0%
Systemic reactions												
Malaise	3	15.0%	3	15.0%	4	20.0%	3	15.0%	1	5.0%	4	20.0%
Headache	3	15.0%	7	35.0%	6	30.0%	1	5.0%	0	0.0%	3	15.0%
Laboratory changes												
Monocyte percentage increased	2	10.0%	1	5.0%	6	30.0%	2	10.0%	2	10.0%	8	40.0%

The severity of adverse events was determined using 4-grade classification systems: the severity of adverse events reported as local reactions (at the administration site) was graded from Grade A to Grade D, and that of adverse events occurring elsewhere from Grade 1 to Grade 4. Grade D and Grade 4 represented the most severe of the 4 grades for each classification system.

Grade C adverse events at the administration site were reported by subjects receiving subcutaneous administration: injection site erythema in 11 subjects, injection site swelling in 8, and injection site pruritus in 1.

Two events of pyrexia were reported as Grade 3 adverse events occurring elsewhere in 2 subjects. The events “disappeared” and were judged to be “unrelated” to the H5N1 Vaccine in both subjects.

Laboratory abnormalities graded as Grade 3 or higher adverse events included 3 events of blood bilirubin increased in 3 subjects and 3 events of blood creatine phosphokinase increased in 3 subjects. The events “disappeared” and were judged to be “unrelated” to the H5N1 Vaccine in both subjects.

There were no deaths or serious adverse events in this study.

Also, no patient discontinued the second administration of the H5N1 Vaccine or the clinical study due to adverse events.

(2) Japanese phase II/III study (Study Number, KIB-PIA02; Attachment, 5.3.5.1-2; Publication, None; Study Period, ■■■ 20■■■ to ■■■ 20■■■)

A multi-center, randomized, double-blind, parallel-group comparative study involving 2 dose groups was conducted at 9 study sites in Japan as an investigator-initiated clinical trial to evaluate the safety and immunogenicity of the H5N1 Vaccine in healthy adults.

The study vaccine preparation (5 or 15 µg in terms of HA content) was to be administered intramuscularly into the upper arm deltoid muscle twice at an interval of 21 ± 7 days.

All 300 vaccinated subjects (5 µg group, 150 subjects; 15 µg group, 150 subjects) were included in the Full Analysis Set (FAS) and also in the safety analysis. Excluding 6 subjects (4 subjects in the 5 µg group and 2 subjects in the 15 µg group) with non-eligibility, protocol violation, and other protocol deviations, the remaining 294 subjects (5 µg group, 146 subjects; 15 µg group, 148 subjects) were included in the Per Protocol Set (PPS).

The primary endpoints of immunogenicity were HI antibody titer against H5 antigen and neutralizing antibody titer against H5N1 influenza virus. The seroconversion rate (the proportion of subjects changing to positive for anti-viral antibodies) was calculated based on the following definition of seroconversion: (a) a post-vaccination HI antibody titer ≥ 20 or ≥ 40 and a four-fold or greater rise in post-vaccination HI antibody titer compared with the baseline level assayed prior to the first administration, and (b) a post-vaccination neutralizing antibody titer ≥ 20 or ≥ 40 and a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration. An antibody titer < 10 was expressed as “5.” The seroconversion rates obtained for the FAS are summarized in Table 7. Two subjects who discontinued the study after the first administration (1 subject in the 5 µg group and 1 subject in the 15 µg group) were excluded from the post-study investigation analysis (investigation at 21 ± 7 days following the second administration).

Table 7: Seroconversion rates (Study KIB-PIA02, FAS)

Dose group	Assessment timing	Number of subjects included	HI antibody titer (equine erythrocytes)				HI antibody titer (chicken erythrocytes)				Neutralizing antibody titer			
			Post-vaccination HI antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level		Post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level		Post-vaccination HI antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level		Post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level		Post-vaccination neutralizing antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level		Post-vaccination neutralizing antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level	
			Number of subjects	Seroconversion rate (95% CI)	Number of subjects	Seroconversion rate (95% CI)	Number of subjects	Seroconversion rate (95% CI)	Number of subjects	Seroconversion rate (95% CI)	Number of subjects	Seroconversion rate (95% CI)	Number of subjects	Seroconversion rate (95% CI)
5 μ g group	Prior to second administration	150	13	8.7% (4.7%-14.4%)	5	3.3% (1.1%-7.6%)	14	9.3% (5.2%-15.2%)	3	2.0% (0.4%-5.7%)	25	16.7% (11.1%-23.6%)	11	7.3% (3.7%-12.7%)
	Post-study investigation	149	43	28.9% (21.7%-36.8%)	11	7.4% (3.7%-12.8%)	7	4.7% (1.9%-9.4%)	1	0.7% (0.0%-3.7%)	97	65.1% (56.9%-72.7%)	38	25.5% (18.7%-33.3%)
15 μ g group	Prior to second administration	150	22	14.7% (9.4%-21.4%)	6	4.0% (1.5%-8.5%)	24	16.0% (10.5%-22.9%)	11	7.3% (3.7%-12.7%)	40	26.7% (19.8%-34.5%)	18	12.0% (7.3%-18.3%)
	Post-study investigation	149	76	51.0% (42.7%-59.3%)	23	15.4% (10.0%-22.3%)	17	11.4% (6.8%-17.6%)	5	3.4% (1.1%-7.7%)	120	80.5% (73.3%-86.6%)	78	52.3% (44.0%-60.6%)

The seroconversion rates in the FAS for HI antibody titer (equine erythrocytes) and neutralizing antibody titer determined at the post-study investigation were higher than those prior to the second administration in both dose groups. Also, the seroconversion rate in the 15 µg group was higher than that in the 5 µg group, regardless of assessment timing (number of administrations experienced) and the type of antibody titer investigated. The seroconversion rates in the PPS showed similar tendencies as noted in the FAS, except that the seroconversion rates for HI antibody titer assayed prior to the second administration (equine erythrocytes, post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise in post-vaccination HI antibody titer compared with the baseline level assayed prior to the first administration) was essentially the same in the 2 dose groups.

The geometric mean titer (GMT) increase was examined as the secondary efficacy endpoint. For HI antibody titer (equine erythrocytes) and neutralizing antibody titer, the GMT increase was proportional to the number of vaccine administrations and tended to be higher in the high-dose group. In contrast, little difference was noted in the GMT increase for the HI antibody titer assayed with chicken erythrocytes, regardless of assessment timing (number of administrations experienced) and the dose of vaccine administration.

Table 8: Geometric mean titer (GMT) increase (Study KIB-PIA02, FAS)

Endpoint	Dose group	Assessment timing	Number of subjects	GMT increase (fold)	
				Geometric mean	Geometric standard deviation
HI antibody titer (equine erythrocytes)	5 µg	Prior to second administration	150	1.27	1.660
		Post-study investigation	149	1.72	2.091
	15 µg	Prior to second administration	150	1.40	1.831
		Post-study investigation	149	2.56	2.203
HI antibody titer (chicken erythrocytes)	5 µg	Prior to second administration	150	1.21	1.642
		Post-study investigation	149	1.10	1.472
	15 µg	Prior to second administration	150	1.48	1.886
		Post-study investigation	149	1.39	1.702
Neutralizing antibody titer	5 µg	Prior to second administration	150	2.13	1.847
		Post-study investigation	149	3.71	2.029
	15 µg	Prior to second administration	150	2.47	1.948
		Post-study investigation	149	5.09	2.046

The severity of adverse events was determined using 4-grade classification systems as in Study KIB-PIA01: the severity of adverse events reported as local reactions (at the administration site) was graded from Grade A to Grade D, and that of adverse events occurring elsewhere from Grade 1 to Grade 4. Grade D and Grade 4 represented the most severe of the 4 grades in each classification system.

A total of 585 adverse events were reported by 214 of 300 subjects (71.3%), including 244 events in 94 of 150 subjects (62.7%) in the 5 µg group and 341 events in 120 of 150 subjects (80.0%) in the 15 µg group. A total of 523 adverse reactions were reported by 196 of 300 subjects (65.3%), including 214 reactions in 83 of 150 subjects (55.3%) in the 5 µg group and 309 reactions in 113 of 150 subjects (75.3%) in the 15 µg group.

No adverse events of Grade 3 or higher were reported in the 5 µg group, while 6 adverse events of Grade 3 or higher (including 5 adverse reactions) were reported in the 15 µg group. No adverse events of Grade 4 were reported in either group.

Tables 9 to 11 summarize adverse events and reactions by dose group: Subjective symptoms/objective signs by System Organ Class and those with an incidence of 3% or higher by Preferred Term (Table 9); local reactions by System Organ Class and by Preferred Term (Table 10); and laboratory abnormalities by Preferred Term reported in at least 2 subjects in either dose group (Table 11).

Table 9: Adverse events and reactions by System Organ Class and those by Preferred Term with an incidence of 3% or higher in either dose group (Subjective symptoms/objective signs) (Study KIB-PIA02, safety analysis set)

System Organ Class/Preferred Term	Adverse events in the 5 µg group (number of subjects, incidence)				Adverse events in the 15 µg group (number of subjects, incidence)			
	Adverse reactions		Adverse events		Adverse reactions		Adverse events	
Number of subjects included	150				150			
Adverse events, number of subjects	27		45		33		49	
Adverse events, incidence	18.0%		30.0%		22.0%		32.7%	
Infections and infestations	1	0.7%	6	4.0%	3	2.0%	9	6.0%
Nasopharyngitis	1	0.7%	3	2.0%	3	2.0%	8	5.3%
Nervous system disorders	6	4.0%	10	6.7%	8	5.3%	10	6.7%
Headache	6	4.0%	9	6.0%	5	3.3%	7	4.7%
Respiratory, thoracic and mediastinal disorders	4	2.7%	10	6.7%	3	2.0%	11	7.3%
Pharyngolaryngeal pain	2	1.3%	4	2.7%	1	0.7%	5	3.3%
Gastrointestinal disorders	1	0.7%	2	1.3%	3	2.0%	5	3.3%
Skin and subcutaneous tissue disorders	2	1.3%	3	2.0%	5	3.3%	7	4.7%
General disorders and administration site conditions	18	12.0%	21	14.0%	22	14.7%	23	15.3%
Malaise	12	8.0%	14	9.3%	19	12.7%	20	13.3%
Pyrexia	4	2.7%	6	4.0%	1	0.7%	1	0.7%

Table 10: Adverse events and reactions by System Organ Class and by Preferred Term (local reactions) (Study KIB-PIA02, safety analysis set)

System Organ Class/Preferred Term	Adverse events in the 5 µg group (number of subjects, incidence)				Adverse events in the 15 µg group (number of subjects, incidence)			
	Adverse reactions		Adverse events		Adverse reactions		Adverse events	
Number of subjects included	150				150			
Adverse events, number of subjects	76		76		108		108	
Adverse events, incidence	50.7%		50.7%		72.0%		72.0%	
Musculoskeletal and connective tissue disorders	6	4.0%	6	4.0%	3	2.0%	3	2.0%
Muscular weakness	6	4.0%	6	4.0%	3	2.0%	3	2.0%
General disorders and administration site conditions	75	50.0%	75	50.0%	107	71.3%	107	71.3%
Injection site bruising	0	0.0%	0	0.0%	1	0.7%	1	0.7%
Injection site erythema	15	10.0%	15	10.0%	21	14.0%	21	14.0%
Injection site induration	0	0.0%	0	0.0%	1	0.7%	1	0.7%
Injection site pain	72	48.0%	72	48.0%	107	71.3%	107	71.3%
Injection site pruritus	9	6.0%	9	6.0%	12	8.0%	12	8.0%
Injection site warmth	8	5.3%	8	5.3%	17	11.3%	17	11.3%
Injection site swelling	8	5.3%	8	5.3%	19	12.7%	19	12.7%

Table 11: Adverse events and reactions by Preferred Term reported in at least 2 subjects in either dose group (laboratory abnormalities) (Study KIB-PIA02, safety analysis set)

System Organ Class/Preferred Term	Adverse events in the 5 µg group (number of subjects, incidence)				Adverse events in the 15 µg group (number of subjects, incidence)			
	Adverse reactions		Adverse events		Adverse reactions		Adverse events	
Number of subjects included	150				150			
Adverse events, number of subjects	1		5		3		6	
Adverse events, incidence	0.7%		3.3%		2.0%		4.0%	
Laboratory abnormalities	1	0.7%	5	3.3%	3	2.0%	6	4.0%
Gamma-glutamyltransferase increased	0	0.0%	1	0.7%	0	0.0%	2	1.3%
White blood cell count increased	0	0.0%	2	1.3%	0	0.0%	1	0.7%

There were no deaths, serious adverse events, or discontinuations of the study due to adverse events.

Outline of review by PMDA

(1) Efficacy

As a result of the regulatory review described below, PMDA has concluded that the immunogenicity of the H5N1 Vaccine against influenza virus strain NIBRG-14 has been sufficiently demonstrated by the submitted study results.

This PMDA conclusion will be further considered, taking Expert Advisors' comments into account.

The details of the efficacy review are described below.

1) Efficacy endpoints

A guideline for seasonal influenza vaccine established by the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products (EMA) (CPMP/BWP/214/96; <http://www.emea.europa.eu/pdfs/human/bwp/021496en.pdf>) requires that at least 1 of the 3 serological assessment criteria for HI titer, as presented in Table 12, be met in assessing the efficacy of influenza vaccine prepared against a particular virus strain recommended once a year for the coming season. In addition, a European Medicines Agency (EMA) guideline on pandemic influenza vaccines coming into effect in 2007 (CHMP/VWP/263499/2006; <http://www.emea.europa.eu/pdfs/human/vwp/26349906enfin.pdf>) requires that all 3 criteria, as defined in the guideline CPMP/BWP/214/96, be fulfilled.

Table 12: EMEA criteria for influenza vaccines (HI antibody titer)

	18-60 years of age	Over 60 years of age
Seroconversion rate	> 40%	> 30%
GMT increase	> 2.5	> 2.0
Response rate	> 70%	> 60%

Seroconversion rate: Proportion of subjects either “with a pre-vaccination HI antibody titer < 10 and a post-vaccination HI antibody titer \geq 40” or “with a four-fold or greater rise in HI antibody titer” (%)

GMT increase: Magnitude of increase in post-vaccination geometric mean titer (GMT) from the pre-vaccination level, with an HI antibody titer below the detection limit (< 10) expressed as “5.”

Response rate: Proportion of subjects with a vaccination HI antibody titer \geq 40 (%)

In Study KIB-PIA02, the applicant defined 2 criteria for seroconversion: (a) a post-vaccination antibody titer \geq 20 and a four-fold or greater rise in post-vaccination antibody titer compared with the baseline level assayed prior to the first administration, or (b) a post-vaccination antibody titer \geq 40 and a four-fold or greater rise in post-vaccination antibody titer compared with the baseline level assayed prior to the first administration. Using these criteria, the applicant assessed the seroconversion rate for HI antibody titer and neutralizing antibody titer as primary endpoints. The applicant assumed in discussing the results of Study KIB-PIA02 that “number of seroconversions or significant increase in antihaemagglutinin antibody titre > 40%,” one of the 3 assessment criteria for HI antibody titer required by the EMEA/CPMP guideline for seasonal influenza vaccines, could be substituted by “subjects with a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration > 40%” in assessment of the H5N1 Vaccine. Then the applicant stated that a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration was confirmed in 97 of 149 subjects (65.1%) in the 5 μ g group and 120 of 149 subjects (80.5%) in the 15 μ g group.* PMDA asked for the applicant’s view on the reasons for such an assessment and discussion of the study results.

The applicant responded as follows.

In Study KIB-PIA02, the seroconversion rate was defined as described above and assessed as the primary endpoint, basically following the EMEA/CPMP guideline. The applicant additionally assessed the seroconversion rate for neutralizing antibody titer, which can be measured at a higher sensitivity than HI antibody titer, and discussed the efficacy of the H5N1 Vaccine preferentially based on this parameter for the following reasons: (a) the EMEA/CPMP guideline provides criteria for HI antibody titer applied to seasonal influenza vaccines; and (b) when used for detection of antibodies produced due to infection with H5N1 influenza virus, the sensitivity of HI antibody titer assay is reportedly inferior to other assay techniques such as neutralizing antibody titer assay and ELISA/western blotting specific to H5 antigen (*J. Clin. Microbiol.*, 1999; 37: 937-943). Substitution of the original assessment criterion according to the EMEA/CPMP guideline “number of seroconversions or significant increase in antihaemagglutinin

* The applicant arbitrarily expressed a neutralizing antibody titer < 10 (under the detection limit) assayed prior to the first administration of the H5N1 Vaccine as “5” and included those with a neutralizing antibody titer of 5 at baseline and a post-vaccination neutralizing antibody titer \geq 20 in “subjects with a four-fold or greater rise in neutralizing antibody titer.”

antibody titre” with “subjects with a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration” can be justified by the fact that “a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration” is generally taken as a significant rise in antibody titer. The validity of a rise in post-vaccination neutralizing antibody titer as an assessment criterion for antibody titer produced by administration of the H5N1 Vaccine may also be supported by the following diagnostic criterion for a confirmed case of human infection with H5N1 influenza virus defined in “WHO case definitions for human infections with influenza A(H5N1) virus” (as of August 29, 2006), “A fourfold or greater rise in neutralization antibody titer for H5N1 based on testing of an acute serum specimen (collected 7 days or less after symptom onset) and a convalescent serum specimen. The convalescent neutralizing antibody titer must also be 1:80 or higher.”

PMDA considers as follows.

Although the associations between immunological indices such as antibody titer and protection against infection/development are not sufficiently clear for pandemic influenza, it is impossible to assess the protective effect of the H5N1 Vaccine against infection with/development of the target disease because no outbreak of pandemic influenza has actually occurred as yet. Accordingly, efficacy of the H5N1 Vaccine must be assessed in terms of its immunogenicity. Considering that a vaccine prepared against H5 influenza virus showed a limited immunogenicity when administered to humans (<http://mhlw-grants.niph.go.jp/niph/search/NIST00.do>, the Study on Safety and Efficacy of Inactivated Whole Virus H5N1 Influenza Vaccine, 2002), the applicant’s claim for validity of the immunogenicity of the H5N1 Vaccine assessed in terms of neutralizing antibody titer that can be assayed at a higher sensitivity compared with HI antibody titer may be understandable. In fact, however, the threshold of neutralizing antibody titer in relation to a protective effect against infection is still unknown even for seasonal influenza vaccines and no efficacy assessment criteria associated with protection against infection are currently available except HI antibody titer incorporated into the EMEA/CPMP guideline on the basis of a reported clinical investigation (*J. Hyg., Camb.*, 1984; 92, 301-312) and empirical association with clinical efficacy (protection against infection). The H5N1 Vaccine differs from seasonal influenza vaccine in that it is intended for primary immunization of populations immunologically naive to pandemic influenza virus, and assessment criteria specified by the EMEA/CPMP guideline for seasonal influenza vaccine may not always be applicable to it. Nevertheless, it is important to assess the results of Study KIB-PIA02 according to the criteria of the EMEA/CPMP guideline. Also, it is necessary to assess HI antibody titer assayed with chicken erythrocytes as well, because the EMEA/CPMP guideline adopts HI antibody titer assayed with avian (chicken or turkey) erythrocytes (*Develop. Biol. Standard.*, 1977; 39: 273-281) as a serological criterion.

In the protocol for Study KIB-PIA02, in terms of the assessment of efficacy, PPS had been defined as the major analysis set for immunogenicity to ensure the methodological consistency with Study KIB-PIA01.

However, prior to locking data sets, the investigator* stated in the statistical analysis plan that the major analysis set for immunogenicity was FAS, without changing the protocol description from PPS to FAS. The investigator justified this change in the analysis set as follows: (a) it was considered valid to define FAS as the major analysis set for the immunogenicity of the H5N1 Vaccine because Study KIB-PIA02 was a confirmatory study; (b) it was considered that changing the analysis set from PPS to FAS would not lead to overestimation of the immunogenicity of the H5N1 Vaccine. PMDA does not consider this change to have seriously affected efficacy assessment in Study KIB-PIA02, because the seroconversion rates in the PPS and FAS were quite comparable. Nevertheless, the protocol should have been revised because definition of the major analysis set is an important issue in clinical study design.

2) Efficacy

PMDA interpreted the definition of seroconversion stated in the EMEA/CPMP guideline (CPMP/BWP/214/96) as either “pre-vaccination HI antibody titer < 10 and post-vaccination HI antibody titer \geq 40” or “pre-vaccination HI antibody titer \geq 10 and a four-fold or greater rise in post-vaccination HI antibody titer compared with the baseline level assayed prior to the first administration.” PMDA then directed the applicant to recalculate seroconversion rates for HI antibody titer according to this alternative definition of seroconversion. Based on the submitted results, PMDA confirmed that the seroconversion rate for HI antibody titer (chicken erythrocytes) at a post-study investigation did not meet the requirement of the EMEA/CPMP guideline (above 40%), as shown in Table 13. PMDA further confirmed that both GMT increase (Table 8) and response rate (Table 14) for HI antibody titer (chicken erythrocytes) failed to fulfill the EMEA/CPMP criteria.

Table 13: Seroconversion rates (according to PMDA’s interpretation) (Study KIB-PIA02, FAS)

Dose group	Assessment timing	Number of subjects included	HI antibody titer (chicken erythrocytes)		HI antibody titer (equine erythrocytes)	
			“With pre-vaccination HI antibody titer < 10 and post-vaccination HI antibody titer \geq 40” or “with pre-vaccination HI antibody titer \geq 10 and a four-fold or greater rise in HI antibody titer”		“With pre-vaccination HI antibody titer < 10 and post-vaccination HI antibody titer \geq 40” or “with pre-vaccination HI antibody titer \geq 10 and a four-fold or greater rise in HI antibody titer”	
			Number of subjects	Seroconversion rate (%) (95% CI)	Number of subjects	Seroconversion rate (%) (95% CI)
5 μ g	Prior to second administration	150	3	2.0 (0.4-5.7)	5	3.3 (1.1-7.6)
	Post-study investigation	149	1	0.7 (0.0-3.7)	11	7.4 (3.7-12.8)
15 μ g	Prior to second administration	150	11	7.3 (3.7-12.7)	6	4.0 (1.5-8.5)
	Post-study investigation	149	5	3.4 (1.1-7.7)	23	15.4 (10.0-22.3)

* Refer to those who conducted the investigator-initiated clinical trial.

Table 14: Antibody response rate (Study KIB-PIA02, FAS)

Endpoint	Dose group	Assessment timing	Number of subjects included	Antibody responses rate	
				Number of subjects	% (95% CI)
HI antibody titer (chicken erythrocytes)	5 µg	Prior to second administration	150	4	2.7 (0.7-6.7)
		Post-study investigation	149	2	1.3 (0.2-4.8)
	15 µg	Prior to second administration	150	11	7.3 (3.7-12.7)
		Post-study investigation	149	5	3.4 (1.1-7.7)
HI antibody titer (equine erythrocytes)	5 µg	Prior to second administration	150	6	4.0 (1.5-8.5)
		Post-study investigation	149	12	8.1 (4.2-13.6)
	15 µg	Prior to second administration	150	8	5.3 (2.3-10.2)
		Post-study investigation	149	26	17.4 (11.7-24.5)

PMDA asked for the applicant's view on the reasons for a smaller increase in HI antibody titer (chicken erythrocytes) generated by the H5N1 Vaccine than that achieved with conventional seasonal influenza vaccines. The applicant responded that the apparently small rise in HI antibody titer (chicken erythrocytes) might be ascribable to the reported lower sensitivity of HI antibody titer assay in detection of antibodies produced due to infection with H5N1 avian influenza virus as compared with other assay techniques such as neutralizing antibody titer assay and ELISA/western blotting specific to H5 antigen (*J. Clin. Microbiol.*, 1999; 37: 937-943).

PMDA further examined the appropriateness of the assay system used for determination of HI antibody titer as follows. In 8 of the 298 subjects receiving the second administration of the H5N1 Vaccine in Study KIB-PIA02, the post-study HI antibody titer (chicken erythrocytes) decreased to a quarter or below of that prior to the second administration (Table 15). PMDA asked for the applicant's view on the cause of this finding.

The applicant responded as follows.

The decrease to a quarter or below in HI antibody titer (chicken erythrocytes) found in the 8 subjects after the second administration of the H5N1 Vaccine might simply be due to non-constant and fluctuating sensitivity of HI antibody titer assay using chicken erythrocytes, because no decrease in neutralizing antibody titer was noted in any of the subjects. Further, taking account of the fact that no such decreases in HI antibody titer (equine erythrocytes) or in neutralizing antibody titer were found in any of the subjects investigated in Study KIB-PIA02, PMDA considers it reasonable to ascribe the decrease in HI antibody titer (chicken erythrocytes) to the assay system used.

Thus, PMDA considers that application of HI antibody titer assayed with chicken erythrocytes, conventionally used as criterion for clinical efficacy of seasonal influenza vaccine, to efficacy assessment of the H5N1 Vaccine would show its limit.

Table 15: List of subjects with a drop in antibody titer to a quarter or below of the pre-vaccination level and changes in antibody titer

(Study KIB-PIA02)

Case No.	Dose group	HI antibody titer (chicken erythrocytes)			Neutralizing antibody titer		
		Prior to first administration	Prior to second administration	Post-study investigation	Prior to first administration	Prior to second administration	Post-study investigation
K09-24	5 µg	5	20	5	5	20	20
K06-35		5	20	5	10	20	20
K06-42		5	20	5	5	10	10
K07-12	15 µg	5	20	5	5	10	20
K06-21		5	20	5	5	10	10
K06-25		5	40	10	5	40	80
K05-14		5	40	10	5	20	80
K04-23		5	40	10	5	10	20

While the EMEA/CPMP guideline for seasonal influenza vaccines consistently uses HI antibody titer assayed with avian (chicken or turkey) erythrocytes as a serological efficacy criterion (*Develop. Biol. Standard.*, 1977; 39: 273-281), an FDA guidance on licensure of pandemic influenza vaccines as of May 2007 (<http://www.fda.gov/cber/gdlns/panfluvac.pdf>) states that not only erythrocytes of avian origin but also those from other animal species may be used in antibody titer assay for efficacy assessment of a pandemic influenza vaccine candidate. Because the relationship between HI antibody titers assayed with erythrocytes of different origin, equine and avian, has not been thoroughly investigated to date, there may still be uncertainty about whether it is reasonable to simply substitute an HI antibody titer assayed with avian erythrocytes ≥ 40 as a criterion for seroprotection level with an HI antibody titer assayed with equine erythrocytes ≥ 40 . However, PMDA considers it reasonable to assume that an HI antibody titer assayed with equine erythrocytes may be equivalent to the value assayed with avian erythrocytes, judging from the principle of HI antibody titer assay. Assuming the equivalence of HI antibody titers assayed with erythrocytes from different species, the GMT increase in HI antibody titer assayed with equine erythrocytes in the 15µg group (2.56) (Table 8) meets the requirement of the EMEA/CPMP guideline, while the seroconversion rate (15.4%) (Table 13) and the response rate (17.4%) (Table 14) are far below the level required by this guideline. PMDA therefore considers it possibly unreasonable to expect protective effects of the H5N1 Vaccine against pandemic influenza virus infection based on the presented HI antibody titer data alone.

The serological criteria required for seasonal influenza vaccines are established by assuming the pre-existence of a certain level of immunity to the influenza virus strain used for vaccine production. The EMEA/CHMP guideline for pandemic influenza vaccines (CHMP/VWP/263499/2006) states that all 3 assessment criteria defined in the preceding EMEA/CPMP guideline should be fulfilled, because pandemic influenza vaccines are intended for primary immunization of immunologically naive populations. Since association between fulfillment of all 3 assessment criteria and achievement of protective effects against virus infection has not been confirmed to date, PMDA considers that the failure to fulfill all 3 assessment criteria required for pandemic influenza vaccines does not necessarily imply

absence of a protective effect against infection with pandemic influenza pathologically different from that with a conventional seasonal influenza.

It may also be necessary to assess immunogenicity of the H5N1 Vaccine in terms of not only HI antibody titer (inhibition indicator of red blood cell agglutination via HA protein) but also neutralizing antibody titer (inhibition indicator of viral adsorption and proliferation), considering that the H5N1 Vaccine is a whole virus vaccine retaining the overall virion structure, but not an HA vaccine with the virion disrupted. In fact, the EMEA/CHMP guideline for pandemic influenza vaccines (CHMP/VWP/263499/2006) states that “a demonstration that the candidate vaccine elicits neutralising antibodies directed against the vaccine strain is very important,” besides requiring fulfillment of all 3 assessment criteria defined in the EMEA/CPMP guideline for seasonal influenza vaccines (CPMP/BWP/214/96).

Whether a correlation exists between neutralizing antibody titer and HI antibody titer (chicken erythrocytes) or between neutralizing antibody titer and a protective effect against influenza virus infection is not clear to date. Accordingly, it is impossible to judge the protective effect of the H5N1 Vaccine against infection based on the submitted study results. Nevertheless, PMDA considers that a vaccine eliciting a four-fold or greater rise in post-vaccination neutralizing antibody titer as compared with the pre-vaccination level may be judged as having a certain clinical significance, because the above-mentioned immunological response suggests an increase in the level of antibodies with a neutralizing activity (i.e., an ability to inhibit viral proliferation) through activation of the immune system by antigenic stimulation equivalent to actual virus infection. PMDA’s view is based on the following points:

- (a) The protective effect of the H5N1 Vaccine against virus infection in humans may well be expected, considering that a neutralizing antibody titer assay measures the ability of antibodies to inhibit viral adsorption and proliferation;
- (b) A four-fold or greater difference in antibody titer between acute-phase and convalescent sera (*Harrison’s Principles of Internal Medicine* 16th ed) is generally accepted as the serological diagnostic criterion for virus infection; and
- (c) “A fourfold or greater rise in neutralization antibody titer for H5N1 based on testing of an acute serum specimen (collected 7 days or less after symptom onset) and a convalescent serum specimen. The convalescent neutralizing antibody titer must also be 1:80 or higher,” which is defined as a diagnostic criterion for a confirmed case of human infection with influenza (H5N1) virus, according to “WHO case definitions for human infections with influenza A(H5N1) virus” (August 29, 2006).

The applicant states that a four-fold or greater rise in neutralizing antibody titer was noted at post-study investigation in Study KIB-PIA02 in 97 of 149 subjects (65.1%) in the 5 µg group and 120 of 149 subjects (80.5%) in the 15 µg group (Table 16). Actually, the applicant arbitrarily expressed a neutralizing antibody titer < 10 (under the detection limit) assayed prior to administration of the H5N1 Vaccine as “5” and included subjects with a neutralizing antibody titer of 5 at baseline and a

post-vaccination neutralizing antibody titer ≥ 20 in “subjects with a four-fold or greater rise in neutralizing antibody titer.” Given such a definition by the applicant, PMDA considers it unreasonable to conclude that all subjects counted in Table 16 as having a four-fold or greater rise in neutralizing antibody titer had, without exception, an actual rise in antibody titer of this magnitude purely as a result of administration of the H5N1 Vaccine. However, the study results also revealed a four-fold or greater rise in neutralizing antibody titer at post-study investigation in subjects with a neutralizing antibody titer ≥ 10 (above the detection limit) assayed prior to administration of the H5N1 Vaccine at a satisfactory rate: 12 of 30 subjects (40.0%) in the 5 μg group and 25 of 34 subjects (73.5%) in the 15 μg group. Based on the discussions described above, PMDA has concluded that the H5N1 Vaccine induced antibody production upon administration to human subjects, revealing the immunogenicity of the H5N1 Vaccine.

Table 16: Proportion of subjects with a four-fold or greater rise in neutralizing antibody titer (Study KIB-PIA02, FAS)

Dose group	Assessment timing	Number of subjects included	Four-fold or greater rise in neutralizing antibody titer compared with the baseline level	
			Number of subjects	% (95% CI)
5 μg	Prior to second administration	150	25	16.7% (11.1-23.6)
	Post-study investigation	149	97	65.1% (56.9-72.7)
15 μg	Prior to second administration	150	40	26.7% (19.8-34.5)
	Post-study investigation	149	120	80.5% (73.3-86.6)

Taken together, neutralizing antibody titer data revealed the induction of antibody production following the administration of the H5N1 Vaccine and the existence of subjects with a rise in HI antibody titer was confirmed in spite of a reportedly low sensitivity of HI antibody titer assay when applied to H5 antigen. Thus, PMDA considers administration of the H5N1 Vaccine to potentially result in acquisition of immunity against pandemic influenza. Highly pathogenic avian influenza (H5N1) is currently prevalent mainly in South-East Asia, with a persistent and expanding epidemic demonstrated by case identification in Europe. Along with growing concern regarding rapid spread into human populations via human-to-human transmission, there is growing expectation that administration of the H5N1 Vaccine will enhance acquisition of immunity against pandemic influenza.

(2) Safety

PMDA considered no serious adverse events to have occurred during the clinical studies of the H5N1 Vaccine and concluded that there are no particular problems with respect to its tolerability based on the submitted safety data, considering that the target disease of the H5N1 Vaccine is extremely serious.

This PMDA conclusion will be finalized, taking Expert Advisors’ comments into account.

The details of the safety review are described below.

In Study KIB-PIA02, comparison between the 2 dose groups demonstrated that the incidence of adverse events (244 events in 94 of 150 subjects [62.7%] in the 5 µg group vs. 341 events in 120 of 150 subjects [80.0%] in the 15 µg group) and adverse reactions (214 events in 83 of 150 subjects [55.3%] in the 5 µg group vs. 309 events in 133 of 150 subjects [75.3%] in the 15 µg group) tended to be higher in the 15 µg group. In addition, a dose-dependent increase was noted not only in local reactions (175 events in 76 of 150 subjects [50.7%] in the 5 µg group vs. 249 events in 108 of 150 subjects [72.0%] in the 15 µg group), consisting of the majority of the adverse events found, but also in systemic reactions as adverse events (64 events in 45 of 150 subjects [30.0%] in the 5 µg group vs. 84 events in 49 of 150 subjects [32.7%] in the 15 µg group) and adverse reactions (38 events in 27 of 150 subjects [18.0%] in the 5 µg group vs. 57 events in 33 of 150 subjects [22.0%] in the 15 µg group). Grade C local reactions occurred only in the 15 µg group (5 reactions in 3 subjects).

As for adverse events by route of administration examined in Study KIB-PIA01, the incidence of local reactions as adverse events was higher in the subcutaneous group (48 of 60 subjects, 80%) than in the intramuscular group (6 of 60 subjects, 10%). A similar tendency was also noted with the incidence of systemic reactions as adverse events: headache, 31.7% (19 of 60 subjects) in the subcutaneous group vs. 16.7% (10 of 60 subjects) in the intramuscular group; and malaise, 30.0% (18 of 60 subjects) in the subcutaneous group vs. 21.7% (13 of 60 subjects) in the intramuscular group. Since the incidence of adverse reactions tended to be higher in the subcutaneous group than in the intramuscular group (Table 17), only intramuscular administration was conducted in Study KIB-PIA02.

**Table 17: Frequency of adverse events by route of administration
(pooled analysis of Studies KIB-PIA01 and KIB-PIA02, safety analysis set)**

	Number of subjects included	Adverse events		Adverse reactions	
		Number of subjects (%)	Number of events	Number of subjects (%)	Number of events
Subcutaneous	60	58 (96.7)	362	54 (90.0)	242
Intramuscular	360	269 (74.7)	764	223 (61.9)	560
Total	420	327 (77.9)	1126	277 (66.0)	802

In Study KIB-PIA02, the number of subjects experiencing the following adverse events upon the first administration differed between the 2 dose groups by 5 or more: malaise (subjective symptoms/objective signs), 6 of 150 subjects (4.0%) in the 5 µg group vs. 15 of 150 subjects (10.0%) in the 15 µg group; injection site pain (local reactions), 64 of 150 subjects (42.7%) in the 5 µg group vs. 92 of 150 subjects (61.3%) in the 15 µg group; and injection site warmth (local reactions), 5 of 150 subjects (3.3%) in the 5 µg group vs. 12 of 150 subjects (8.0%) in the 15 µg group.

Upon the second administration, the number of subjects experiencing the following 4 adverse events, all belonging to the category of local reactions, differed between the 2 dose groups by 5 or more: injection site pain, 51 of 149 subjects (34.2%) in the 5 µg group vs. 72 of 149 subjects (48.3%) in the 15 µg group;

injection site erythema, 6 of 149 subjects (4.0%) in the 5 µg group vs. 12 of 149 subjects (8.1%) in the 15 µg group; injection site warmth, 3 of 149 subjects (2.0%) in the 5 µg group vs. 8 of 149 subjects (5.4%) in the 15 µg group; and injection site swelling, 4 of 149 subjects (2.7%) in the 5 µg group vs. 11 of 149 subjects (7.4%) in the 15 µg group. For all these adverse events, the incidence was higher in the 15 µg group.

Comparison of adverse events assessed after the first and second administrations revealed that both subjective symptoms/objective signs and local reactions tended to show a higher incidence at first administration in both dose groups (Table 18).

Table 18: Frequency of adverse events by administration timing in Study KIB-PIA02 (safety analysis set, modified by PMDA from the submitted data)

Dose group		First administration			Second administration		
		Number of subjects included	Number of subjects	Incidence	Number of subjects included	Number of subjects	Incidence
5 µg	Overall	150	82	54.7%	149	72	48.3%
	Subjective symptoms/objective signs		27	18.0%		25	16.8%
	Local reactions		69	46.0%		55	36.9%
15 µg	Overall	150	103	68.7%	149	82	55.0%
	Subjective symptoms/objective signs		36	24.0%		29	19.5%
	Local reactions		93	62.0%		73	49.0%
Adverse reactions							
Dose group		First administration			Second administration		
		Number of subjects included	Number of subjects	Incidence	Number of subjects included	Number of subjects	Incidence
5 µg	Overall	150	75	50.0%	149	64	43.0%
	Subjective symptoms/objective signs		15	10.0%		17	11.4%
	Local reactions		69	46.0%		55	36.9%
15 µg	Overall	150	96	64.0%	149	77	51.7%
	Subjective symptoms/objective signs		23	15.3%		20	13.4%
	Local reactions		93	62.0%		73	49.0%

When multiple adverse events occurred in one subject, the event most strongly associated with administration of the H5N1 Vaccine was counted. Incidence (%) = (Number of subjects developing an adverse event/Number of subjects included in analysis) × 100

The applicant responded as to the reason for the higher incidence of adverse events upon the first administration of the H5N1 Vaccine as follows.

No major problems were noted with adverse events occurring upon the first administration, nor were

serious adverse events identified on this occasion. Also, none of these adverse events tended to worsen with time after the first administration. Accordingly, there may be no need for special measures to cope with adverse events occurring after the first administration, despite their relatively high incidence.

PMDA confirmed that adverse events occurred more frequently upon the first than the second administration, except for malaise in the 5 µg group (Tables 19 to 22). Although adverse events were noted at high frequencies, no serious adverse reactions actually occurred. Considering that the target disease of the H5N1 Vaccine is extremely serious, PMDA concluded that there are no particular problems with respect to its tolerability based on the submitted safety data.

**Table 19: Incidence of major adverse events by administration timing in Study KIB-PIA02
(5 µg group, safety analysis set)**

Category	SOC/PT	First administration			Second administration		
		Number of subjects included	Number of subjects	Incidence (%)	Number of subjects included	Number of subjects	Incidence (%)
Subjective/symptoms objective signs	Nervous system disorders	150			149		
	Headache		7	4.7		3	2.0
	General disorders and administration site conditions						
Malaise	6		4.0	8		5.4	
Local reactions	General disorders and administration site conditions						
	Injection site erythema		15	10.0		6	4.0
	Injection site pain		64	42.7		51	34.2
	Injection site pruritus	7	4.7	5	3.4		
	Injection site warmth	5	3.3	3	2.0		
	Injection site swelling	7	4.7	4	2.7		

**Table 20: Incidence of major adverse events by administration timing in Study KIB-PIA02
(15 µg group, safety analysis set)**

Category	SOC/PT	First administration			Second administration		
		Number of subjects included	Number of subjects	Incidence (%)	Number of subjects included	Number of subjects	Incidence (%)
Subjective symptoms/ objective signs	Nervous system disorders	150			149		
	Headache		5	3.3		2	1.3
	General disorders and administration site conditions						
Malaise	15		10.0	9		6.0	
Local reactions	General disorders and administration site conditions						
	Injection site erythema		13	8.7		12	8.1
	Injection site pain		92	61.3		72	48.3
	Injection site pruritus	10	6.7	3	2.0		
	Injection site warmth	12	8.0	8	5.4		
	Injection site swelling	11	7.3	11	7.4		

Table 21: Incidence of major adverse reactions by administration timing in Study KIB-PIA02

(5 µg group, safety analysis set)

Category	SOC/PT	First administration			Second administration		
		Number of subjects included	Number of subjects	Incidence (%)	Number of subjects included	Number of subjects	Incidence (%)
Subjective symptoms/ objective signs	Nervous system disorders	150			149		
	Headache		4	2.7		3	2.0
	General disorders and administration site conditions						
	Malaise		5	3.3		7	4.7
Local reactions	General disorders and administration site conditions	150			149		
	Injection site erythema		15	10.0		6	4.0
	Injection site pain		64	42.7		51	34.2
	Injection site pruritus		7	4.7		5	3.4
	Injection site warmth		5	3.3		3	2.0
	Injection site swelling		7	4.7		4	2.7

Table 22: Incidence of major adverse reactions by administration timing in Study KIB-PIA02

(15 µg group, safety analysis set)

Category	SOC/PT	First administration			Second administration		
		Number of subjects included	Number of subjects	Incidence (%)	Number of subjects included	Number of subjects	Incidence (%)
Subjective symptoms/ objective signs	Nervous system disorders	150			149		
	Headache		3	2.0		2	1.3
	General disorders and administration site conditions						
	Malaise		14	9.3		9	6.0
Local reactions	General disorders and administration site conditions	150			149		
	Injection site erythema		13	8.7		12	8.1
	Injection site pain		92	61.3		72	48.3
	Injection site pruritus		10	6.7		3	2.0
	Injection site warmth		12	8.0		8	5.4
	Injection site swelling		11	7.3		11	7.4

Compared with local reactions associated with seasonal influenza vaccine (11.4% according to the package insert of Influenza HA Vaccine “S Hokken” “JP,” revised in July 2006 [fifth Edition]), local reactions associated with the H5N1 Vaccine tend to occur more frequently (Study KIB-PIA02, 50.7% [76 of 150 subjects] in the 5 µg group and 72.0% [108 of 150 subjects] in the 15 µg group). The applicant ascribed this to (a) the difference in methods for collection of adverse events data and (b) use of aluminium hydroxide as an adjuvant. PMDA considers the applicant’s explanation to be acceptable.

Since serious adverse reactions associated with seasonal influenza vaccine include shock or anaphylactoid symptoms, acute disseminated encephalomyelitis (ADEM), Guillain-Barre syndrome, convulsion (including febrile convulsion), hepatic dysfunction, jaundice, and asthmatic attacks, PMDA considers that information on such adverse reactions should be carefully collected after marketing.

(3) Clinical positioning

While reported cases of influenza suspected to be via human-to-human transmission are extremely rare at present except for those caused by H1N1 and H3N2 subtype pandemic strains (*N. Engl. J. Med.*, 2005; 352: 333-340, *J. Infect. Dis.* 2000; 181: 344-348), the total number of confirmed human cases of influenza (H5N1) available from the WHO website is 315 including 191 deaths (as of June 25, 2007), which demonstrates persistently high fatality with this pathogen in humans and a steady annual increase in both patients and deaths (Table 23; http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_06_15/en/index.html). The fatality rate documented for “Spanish flu” virus (H1N1) was 2.5% or higher during the 1918-1919 pandemic (Marks, G. & Beatty, W. K. *Epidemics.*, 1976; New York: Scribner.). The lethality of the present H5N1 strain may be reduced in a possible future human pandemic; however, recent serologic surveys in Vietnam and Thailand have revealed no evidence of asymptomatic infections (*N. Engl. J. Med.*, 2005; 353: 1374-1385). Therefore, it is currently anticipated that human infection with a pandemic influenza virus strain derived from the avian influenza virus A/H5N1 strains will involve more severe symptoms.

Table 23: Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) reported to WHO (as of June 25, 2007)

	2003	2004	2005	2006	2007	Total
Cases	4	46	98	115	52	315
Deaths	4	32	43	79	33	191
Fatality (%)	100.0	69.6	43.9	68.7	63.5	60.6

As of the time of this regulatory review, the following information has become available on possible treatment methods for pandemic influenza (H5N1), along with their efficacies.

Oseltamivir and zanamivir are reportedly effective in animal models infected with H5N1 influenza virus (*J. Infect. Dis.*, 1998; 178: 1592-1596, *Antimicrob. Agents Chemother.*, 2001; 45: 1216-1224, *Nature*, 2005; 437: 1108). These antivirals have also been reported to inhibit proliferation of H5N1 influenza virus strains isolated from infected humans, suggesting their prophylactic and therapeutic efficacy (*J. Infect. Dis.*, 1998; 178: 1592-1596, *Antimicrob. Agents Chemother.*, 2001; 45: 1216-1224, *Antimicrob. Agents Chemother.*, 2001; 45: 743-748, *J. Infect. Dis.*, 2005; 192: 665-672). In fact, however, their NA-inhibiting and antiviral activities *per se* are not extraordinarily high. Of additional concern, 95% or more of H5N1 influenza virus strains isolated in Vietnam and Thailand were reportedly resistant to amantadine and rimantadine (*J. Infect. Dis.*, 2006; 193: 1626-1629).

The following reports are available on clinical use of oseltamivir. Out of 8 patients with H5N1 influenza receiving oseltamivir, 4 died and oseltamivir-resistant virus strains were isolated from 2 of them (*N. Engl. J. Med.*, 2005; 353: 2667-2672). Antiviral treatment with oeltamivir in patients with H5N1 influenza identified in Vietnam in 2004 led to the following outcomes: H5N1 influenza virus had disappeared from clinical samples within 2 or 3 days after initiation of oseltamivir administration in surviving patients,

while early initiation of oseltamivir administration in patients who died had failed to stop disease progression or sufficiently reduce viral count in the pharynx (*Emerg. Infect. Dis.*, 2005; 11: 201-209, *N. Engl. J. Med.*, 2004; 350: 1179-1188).

Judging from the above-mentioned findings, treatment methods for human infection with H5N1 influenza virus are still extremely uncertain and far from established, although some successful attempts have been reported. Therefore, PMDA considers prophylaxis to be most important for possible countermeasures against pandemic influenza virus infection with an anticipated high fatality rate in humans. Since the immunogenicity of the H5N1 Vaccine in humans was demonstrated, as discussed under “(1) Efficacy” and a similar vaccine containing inactivated whole influenza virions as the active ingredient was confirmed to show a protective effect against influenza virus infection in mice [see “3. Non-clinical data, *Outline of review by PMDA*, Protective effect of the H5N1 Vaccine against infection”], the PMDA considers administration of the H5N1 Vaccine potentially reduce clinical symptoms and decreases fatality associated with pandemic influenza infection.

The Pandemic Influenza Preparedness Action Plan of the Japanese Government (hereinafter referred to as “Action Plan”) states that, at WHO Phase 4 when human-to-human transmission of a new subtype of influenza virus is confirmed, the following countermeasures will be implemented: cooperation with reference laboratories of WHO, World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) for identification and analysis of the virus strain, acquisition of the virus subtype in question, development of candidate virus strains for production of vaccines, and commencement of manufacturing of pandemic vaccines in Japan. PMDA asked the applicant to roughly estimate how long it will take from obtaining the master seed to production of the first vaccine lot. The applicant responded that it will take approximately 3 months (seed lot preparation: [REDACTED] days, fermentation: [REDACTED] weeks, formulation: [REDACTED] days, specification testing: [REDACTED] months).

Although the first wave of the “Spanish flu” pandemic (1918-1919) was highly contagious but not especially fatal, the second wave was characterized by a 10-fold increase in the fatality rate. In the “Asian flu” pandemic in 1957-1958, a second wave occurred 2 to 3 month after the disappearance of the first wave, causing increased fatalities. PMDA judges that, based on the applicant’s response and experiences of the previous influenza pandemics mentioned above, the causative virus strain isolated during the first wave of a future pandemic can be used for production of a prophylactic vaccine in time for the onset of the second wave considered to be associated with higher fatality.

Historically, the milder clinical symptoms and lower fatality associated with the “Hong Kong flu” pandemic (1968-1969) as compared with those associated with “Spanish flu” (1918-1919) and “Asian flu” (1957-1958) pandemics have been at least partly ascribed to the fact that the immediately preceding “Asian flu” pandemic was caused by the H2N2 strain sharing the N2 subtype with influenza virus A/H3N2, the causative agent of the subsequent “Hong Kong flu” pandemic, and immunity against N2

antigen acquired during the former pandemic protected the exposed populations from the subsequent infection with the H3N2 strain (*J. Infect. Dis.*, 2005; 192: 233-248). Since a similar vaccine containing inactivated whole influenza virions as the active ingredient was confirmed to exhibit cross-protection against H5N1 influenza virus strains in mice [see “3. Non-clinical data, *Outline of review by PMDA, Protective effect of the H5N1 Vaccine against infection*”], PMDA considers administration of the H5N1 Vaccine to potentially reduce clinical symptoms and decrease fatality associated with pandemic influenza infection even when the virus strain used for vaccine production is not identical to that causing the actual pandemic.

The Action Plan states that, at WHO Phase 3 when human infection with a new subtype of influenza virus is confirmed, the Japanese Ministry of Health, Labour and Welfare will review candidate virus strains for production of pre-pandemic vaccines according to the availability of clinical isolates of highly pathogenic avian influenza virus at risk for causing a pandemic in humans in order to start manufacturing and stockpiling pre-pandemic vaccine sources. Although the efficacy of the H5N1 Vaccine in actual pandemic influenza infection remains uncertain due to antigenic variation of the causal virus, infection prophylaxis by administration of the H5N1 Vaccine is currently expected to serve as an emergency countermeasure at the pre-pandemic stage (the period prior to the first wave of a pandemic, WHO Phases 4 and 5) and against the first wave of the pandemic.

Judging from the non-clinical and clinical data submitted and based on the historical experiences mentioned above, PMDA considered the H5N1 Vaccine to potentially exhibit a protective effect against pandemic influenza or prevent symptoms becoming more severe, and thus concluded that the H5N1 Vaccine should be positioned as a prophylactic vaccine against pandemic influenza virus infection.

This PMDA conclusion will be finalized, taking Expert Advisors’ comments into account.

(4) Indications

Based on the data for the H5N1 Vaccine manufactured using influenza virus strain NIBRG-14 and also on the discussion described under “(3) Clinical positioning,” PMDA has concluded that it is acceptable to state in the INDICATIONS section that the vaccine is indicated for “prophylaxis of pandemic influenza.” However, the following measures are considered to be necessary. Since no data on efficacy and safety of the H5N1 Vaccine in younger subjects (under 20 years of age) and elderly people (65 years of age or older) were submitted for this regulatory review, it is appropriate to include the description “Immunogenicity and safety have not been established in pediatric and geriatric populations.” in the PRECAUTIONS section in the package insert of the product. Also, assessing immunogenicity and safety in children and elderly people after marketing is essential, as discussed below [see “(6) Post-marketing considerations”].

PMDA asked the applicant to specify which individuals, if any, are not eligible for immunization with the

H5N1 Vaccine.

The applicant responded as follows.

As is the case with vaccination against seasonal influenza, individuals falling under Paragraphs 2 to 4 and 6, Article 2 of Enforcement Regulations of Preventive Vaccination Law in Japan (Paragraph 2, Individuals with apparent pyrexia; Paragraph 3, Individuals evidently developing a severe acute disease; Paragraph 4, Individuals with an obvious history of anaphylactic reaction to any ingredient of an injection used for immunization against the target disease; and Paragraph 6, Individuals not falling under Paragraphs 2 to 5 but in a condition not eligible for immunization) may not be eligible for immunization with vaccines including the H5N1 Vaccine. Considering that the target disease of the H5N1 Vaccine is extremely serious, however, such individuals should not be excluded from the subject population of the investigational vaccine. It may be desirable to include the following statement in the package insert of the product: “The vaccine should be used with caution, with consideration of health status and constitution of the recipient, after careful consultation and assessment of eligibility for vaccination followed by fully informing the recipient of the necessity, adverse reactions, and usefulness of vaccination to secure prior consent of the recipient, and only if the potential benefits outweigh the potential risks.”

PMDA accepted the applicant’s response.

This PMDA conclusion will be further considered, taking Expert Advisors’ comments into account.

(5) Dosage and administration

The proposed DOSAGE AND ADMINISTRATION is “The dosage is a single injection of 0.5 mL per dose or 2 doses approximately 3 weeks apart, administered intramuscularly or subcutaneously.”

PMDA has concluded that, based on the clinical study data, the DOSAGE AND ADMINISTRATION should be “The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses,” with the antigen content per dose (0.5 mL) specified as “15 µg (in terms of HA antigen)” in the columns of Ingredients and Their Quantity and Manufacturing Method in the approval certificate.

This PMDA conclusion will be finalized, taking Expert Advisors’ comments into account.

The details of the review are described below.

1) Route of administration

PMDA asked for the applicant’s view on the reasons for proposing both subcutaneous and intramuscular injections as routes of administration of the H5N1 Vaccine although Study KIB-PIA02 involved

intramuscular injection exclusively.

The applicant responded as follows.

Since all vaccinations in Japan currently involve subcutaneous injection, it would be preferable to include this as an alternative route of the H5N1 Vaccine administration to avoid possible confusion in clinical practice. Although Study KIB-PIA01 demonstrated that subcutaneous injection tended to be associated with adverse reactions at higher frequencies than intramuscular injection, no serious or remarkable adverse reactions were noted concomitantly and acquisition of an ability to produce antibodies was confirmed after subcutaneous injection. This led to the conclusion that the benefit of prophylactic effects associated with subcutaneous injection of the H5N1 Vaccine would outweigh the risk of adverse reactions at higher frequencies, the basis for proposal of both subcutaneous and intramuscular injections as routes of administration.

PMDA considers as follows.

Study KIB-PIA01 demonstrated that the frequency of adverse events was higher in the subcutaneous than the intramuscular administration group [see “(2) Safety”]. However, as the applicant responded, there were no serious adverse events or discontinuations of the second administration of the H5N1 Vaccine or the clinical study due to adverse events. Also, Grade 3 and Grade C or higher adverse events eventually resolved. Accordingly, there is no need or reason for rejecting subcutaneous injection, considering the seriousness of the target disease and situations in clinical practice anticipated when the H5N1 Vaccine is used. Thus, it is reasonable to provide information on the availability of both subcutaneous and intramuscular injections as routes of administration with the latter yielding the higher antibody titer and to state in the PRECAUTIONS section that “Experiences with subcutaneous injection are limited.”

Table 24: Assessment of neutralizing antibody titer against H5N1 influenza virus (Study KIB-PIA01, PPS)

	Subcutaneous injection, % (subjects)			Intramuscular injection, % (subjects)		
	1.7 µg	5 µg	15 µg	1.7 µg	5 µg	15 µg
Seroprotection rate (post-study investigation)	10.0 (2/20)	42.1 (8/19)	52.6 (10/19)	35.0 (7/20)	65.0 (13/20)	88.9 (16/18)
Four-fold or greater rise (post-study investigation)	25.0 (5/20)	57.9 (11/19)	73.7 (14/19)	50.0 (10/20)	75.0 (15/20)	100.0 (18/18)

2) Antigen content per dose

PMDA considers as follows.

It is desirable to specify the antigen content per dose of the H5N1 Vaccine as 15 µg, expecting a rise in antibody titer to the extent possible, because the threshold antibody titer in relation to the protective effect against infection is unknown. Furthermore, as mentioned in “(2) Safety,” Study KIB-PIA02 demonstrated a dose-dependent increase in the incidences of both adverse events and adverse reactions but they appeared tolerable, considering that the target disease of the H5N1 Vaccine is extremely serious. Thus, there should be no particular problems in view of safety with defining the antigen content of the H5N1 Vaccine as 15 µg per dose, the highest dose assessed in the clinical studies.

3) Dosage and administration

While the proposed DOSAGE AND ADMINISTRATION is “The dosage is a single injection of 0.5 mL per dose or 2 doses approximately 3 weeks apart, administered intramuscularly or subcutaneously,” the applicant explained that the H5N1 Vaccine should be administered in actual use according to the dosage and administration recommended by authorities based on factors such as the current phase of the influenza pandemic, the available vaccine supply, and the Action Plan (or governmental policy). The applicant ascribed this to possible inability to produce sufficient amounts of vaccine and difficulty of multiple visits to medical institutions for vaccination due to restrictions on social activities, both anticipated upon pandemic onset.

PMDA considers as follows.

As described in “2) Antigen content per dose,” it is desirable to specify the antigen content per dose of the H5N1 Vaccine as 15 µg and the dosage as 2 injections, expecting a rise in antibody titer to the extent possible with the threshold of antibody titer in relation to the protective effect against infection unknown.

However, it may become impossible to ensure a sufficient production of the H5N1 Vaccine at the onset of a pandemic, as the applicant stated. Furthermore, social activities of the population will be restricted in association with such a pandemic (Action Plan), and thus, it may become difficult for individuals to receive multiple injections of the H5N1 Vaccine. As suggested from the experience of “Hong Kong flu” pandemic (*J. Infect. Dis.*, 2005; 192: 233-248), it is assumed there would arise the necessity of examining a possible strategy, which allows immunization with a virus strain causing the pandemic to prevent symptoms becoming more severe, despite the reduction of the antigen content per dose. PMDA therefore considers that 1 or 2 injections of the H5N1 Vaccine at an antigen content of 5 to 15 µg per dose may be acceptable depending on the situation, but that further assessment of administration at a reduced dose is desired [see “(6) Post-marketing considerations, 3) Considerations for antigen saving”].

(6) Post-marketing considerations

1) Populations not included in clinical studies

a. Children

A WHO report (*Weekly epidemiological record.*, No. 26, 2007, 82, 41-48) analyzed 256 cases of human infection with H5N1 influenza virus strain reported between November 25, 2003 and November 24, 2006 and demonstrated the highest patient number and fatality rate to be in the age group between 10 and 19 years (Table 25).

Table 25: Fatality rate among laboratory-confirmed human cases of H5N1 influenza infection by age group

(Source: *Weekly epidemiological record.*, No. 26, 2007, 82, 41-48)

Age group (years)	< 5	5-9	10-19	20-29	30-39	40-49	≥ 50	Total
Fatality rate (%)	44.4 (12/27)	48.7 (19/39)	75.8 (50/66)	63.0 (34/54)	65.9 (27/41)	42.9 (6/14)	40.0 (6/15)	60.2 (154/256)

Based on the WHO data suggesting the possibility of a high incidence of infection and a high fatality rate in the younger population upon the onset of pandemic influenza, and considering previous reports on seasonal influenza indicating that (a) the risk of death and sequelae due to influenza-related encephalopathy tended to be higher in the younger population; (b) an increase in excess mortality in elderly people (*N. Engl. J. Med.*, 2001; 344: 889-896) and a rapid increase in influenza mortality in infants (*Nippon Eiseigaku Zasshi [Japanese Journal of Hygiene]* 2002; 57: 571-584) were noted after discontinuation of mass vaccination of schoolchildren in the 1980's; (c) vaccination is effective in preventing encephalopathy (Steering Committee of the Japanese Society for Pediatric Infectious Diseases, *Shoni Kansen Meneki [Infection and Immunity in Childhood]*. 1999; 11: 429-431); and (d) children constitute a vulnerable population (the clinical trial directive EU/2001/20 Official J. European Communities 1.5.2001 L 121/34), PMDA considers development of the H5N1 Vaccine for recipients 20 years of age or younger to be essential and advocate that it be conducted as soon as possible.

PMDA asked the applicant about the development schedule for the H5N1 Vaccine in children. The applicant responded that it would be extremely difficult to put into practice while admitting that the efficacy and safety of the H5N1 Vaccine should be confirmed in children.

Administration of the H5N1 Vaccine in children is not recommended because no pediatric clinical data, particularly information on safety, was submitted for this regulatory review; however, PMDA has concluded that children should not be excluded from the target population of the H5N1 Vaccine and lack of sufficient information on safety in children should be clearly stated in the "Pediatric Use" section of the package insert, considering that the target disease of the H5N1 Vaccine is extremely serious. On the other hand, PMDA considers that detailed post-marketing information on immunogenicity and safety in children should be collected, and in particular, that the safety of the H5N1 Vaccine should be assessed as soon as possible.

b. Elderly people

Study KIB-PIA02 demonstrated that the proportion of subjects with a four-fold or greater rise in neutralizing antibody titer tended to be higher in the younger age group (20-40 years) than in the older age group (40-65 years) (Table 26). Based on this finding, PMDA asked for the applicant's view on the effect of age on the efficacy of the H5N1 Vaccine.

The applicant responded that, although no apparent difference in seroconversion rate* was noted between the 2 age groups, the efficacy of the H5N1 Vaccine may possibly be affected by the age of the recipient, considering the general tendency for a smaller post-vaccination rise in antibody titer in elderly people as compared with that in younger adults (*Vaccine*, 2006; 24: 1159-1169).

* A post-vaccination antibody titer ≥ 20 with a four-fold or greater rise compared with the baseline pre-vaccination level.

Table 26: Proportion of subjects with a post-vaccination neutralizing antibody titer ≥ 20 and a four-fold or greater rise compared with the baseline pre-vaccination level by age (Study KIB-PIA02)

Dose group	Assessment timing	≥ 20 years, < 40 years			≥ 40 years, < 65 years		
		Number of subjects included	Neutralizing antibody titer ≥ 20 and a four-fold or greater rise		Number of subjects included	Neutralizing antibody titer ≥ 20 and a four-fold or greater rise	
			Number of subjects	(%)		Number of subjects	(%)
5 μ g	Prior to second administration	95	16	16.8	55	9	16.4
	Post-study investigation	94	66	70.2	55	31	56.4
15 μ g	Prior to second administration	88	22	25.0	62	18	29.0
	Post-study investigation	88	73	83.0	61	47	77.0

PMDA accepted the applicant’s response and concluded as follows.

Although administration of the H5N1 Vaccine in elderly people is not recommended because no safety data for this population is currently available, elderly people should not be excluded from the target population of the H5N1 Vaccine. Also, lack of sufficient information on safety in elderly people should be clearly stated in the “Use in the Elderly” section of the package insert, considering that the target disease of the H5N1 Vaccine is extremely serious. In addition to the above measure, it is necessary to quickly collect post-marketing information on the immunogenicity and safety of the H5N1 Vaccine in elderly people.

c. Women of child-bearing age

PMDA asked the applicant to explain clinical and non-clinical data currently available on the safety of the H5N1 Vaccine in women of child-bearing age and in pregnant women.

The applicant responded as follows.

During Study KIB-PIA02, a female subject was found to be pregnant. She received 2 injections of the investigational vaccine, experiencing injection site pain (the day following the first administration and the day of the second administration) and pyrexia (14 days after the second administration) as adverse events. Her outcome is currently being checked. A 4-week repeat-dose subcutaneous toxicity study (Segment I, extrapolated as study on fertility and early embryogenesis up to implantation) and a reproductive and developmental toxicity study (Segment III, study on embryonic and fetal development) demonstrated no findings suggesting reproductive or developmental toxicity of the H5N1 Vaccine. In addition, an interim analysis report on an ongoing reproductive and developmental toxicity study (Segment II, study on pre- and postnatal development and maternal functions) identified no changes suggesting an effect of administration of the H5N1 Vaccine. Although this non-clinical data suggests that effects of administration of the H5N1 Vaccine in women of child-bearing age and in pregnant women on maternal health may be minor, sufficient clinical results of the H5N1 Vaccine have not yet been

accumulated and it should therefore be administered to such recipients after careful assessment of its benefits according to the phase of the actual influenza pandemic. This conclusion will be finalized, taking the final results of the ongoing study on pre- and postnatal development and maternal functions into account.

PMDA accepted the applicant's response.

2) Efficacy and safety of the H5N1 Vaccine manufactured with a vaccine strain other than NIBRG-14

The applicant submitted for the regulatory review the data on the quality, efficacy (immunogenicity), and safety of the H5N1 Vaccine manufactured with strain NIBRG-14 as a mock-up vaccine against pandemic influenza. However, the efficacy and safety of the H5N1 Vaccine manufactured with a vaccine strain derived from another pandemic influenza virus strain, including those of the H5N1 serotype, have not been investigated. Therefore, it remains obscure whether the efficacy and safety of such a vaccine are identical to those of the original H5N1 Vaccine manufactured with strain NIBRG-14 or how much these vaccines differ, if at all, with respect to efficacy and safety. PMDA considers it necessary to confirm the efficacy (immunogenicity) and safety of the H5N1 Vaccine manufactured with a different vaccine strain in place of strain NIBRG-14 in the near future.

3) Considerations for antigen saving

PMDA has concluded that, based on the clinical study data, the DOSAGE AND ADMINISTRATION should be "2 injections of 15 µg HA per dose" [see "(5) Dosage and administration"].

The H5N1 Vaccine is manufactured by purifying and inactivating virus propagated in embryonated chicken eggs and production of the vaccine antigen depends on the number of chicken eggs available. The possible prevalence of highly pathogenic avian influenza in chickens concomitant with an outbreak of pandemic influenza in humans is a situation in which securing a sufficient number of chicken eggs for vaccine production is assumed to be difficult or even impossible. When the amount of available vaccine antigen is limited, reducing the antigen content per dose to increase the number of vaccine recipients may achieve a greater protective effect against pandemic influenza virus infection at the population level at the onset of the pandemic, a strategy preferable in view of public health rather than immunizing a limited number of recipients according to the pre-defined DOSAGE AND ADMINISTRATION. The Japanese Ministry of Health, Labour and Welfare plans to take hold of the vaccine supply and discuss the priority for vaccination at the onset of an actual pandemic ("Guidelines on Vaccination against Pandemic Influenza"). The submitted clinical data demonstrates that the H5N1 Vaccine induced antibody production in humans when administered twice at a dose of 5 µg antigen per injection, although with a smaller rise in antibody titer compared with that achieved when administered twice at a dose of 15 µg of antigen per injection. This finding suggests that administration of the H5N1 Vaccine with the antigen content per dose reduced from 15 µg in an actual influenza pandemic may exhibit a certain priming

effect. PMDA therefore considers further assessment of administration of the H5N1 Vaccine at a reduced dose to be desired, including clinical studies to confirm a booster effect of re-vaccination after a certain period following the initial low-dose vaccination.

4) Cross-protective effect

As described earlier, a vaccine similar to the H5N1 Vaccine that contains inactivated whole influenza virions as the active ingredient was confirmed to exhibit cross-protection against H5N1 influenza virus strains with different antigenicity in mice [see “3. Non-clinical data, *Outline of review by PMDA, Protective effect of the H5N1 Vaccine against infection*”], which suggests that administration of the H5N1 Vaccine has the potential to reduce clinical symptoms and decrease fatalities associated with pandemic influenza infection even when the virus strain used for vaccine production is not identical to that causing the actual pandemic. PMDA considers it desirable to further assess the cross-reactivity of antibodies induced by administration of the H5N1 Vaccine in humans with influenza virus strains other than that used for vaccine production.

III. Results of Compliance Review Concerning the Documents Appended to the New Drug Application and Conclusion by PMDA

1. PMDA conclusion regarding the results of document compliance review

Document compliance review was conducted in accordance with the provisions of the Pharmaceutical Affairs Law for documents appended to the new drug application. As there were no major problems, it was concluded that there should be no problem with conducting a regulatory review based on the application dossier.

2. PMDA conclusion regarding the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Law for the documents appended to the new drug application (Study [REDACTED], 5.3.5.1-1 and Study [REDACTED], 5.3.5.1-2). The results revealed: failure to submit the audit protocol to the head of the study site, deficiencies in records concerning the quality and control of the investigational vaccine, inadequate monitoring activities, non-compliance with the procedure for preparation of the clinical study reports ([REDACTED]), and deficiencies concerning management of the Institutional Review Board of some study sites related to the audit report and monitoring report. However, as there were no major problems, it was concluded that no inconvenience would arise in conducting a regulatory review based on the application dossier.

IV. Overall Evaluation

As for the efficacy of the H5N1 Vaccine, although it is difficult at present to assess its protective effect against pandemic influenza infection, the H5N1 Vaccine induced, after 2 injections at a dose of 15 µg per

injection, an increase in HI antibody titer (equine erythrocytes) fulfilling only 1 of these 3 assessment criteria, all of which should be fulfilled according to the EMEA/CHMP guideline for pandemic influenza vaccines (CHMP/VWP/263499/2006). However, the magnitude of increase in neutralizing antibody titer seen concomitantly suggested the occurrence of an immunological response that might be of clinical significance. PMDA therefore considers the administration of the H5N1 Vaccine to potentially result in acquisition of immunity against pandemic influenza. Also, a whole virus influenza vaccine manufactured with strain NIBRG-14 according to a procedure similar to that of the H5N1 Vaccine and formulated in an identical manner was confirmed to show a protective effect against challenge with a highly virulent H5N1 influenza virus strain in mice. Based on these findings, PMDA has concluded that the H5N1 Vaccine has the potential to exhibit a protective effect against pandemic influenza virus infection or prevent symptoms becoming more severe.

As for the safety of the H5N1 Vaccine, adverse reactions occurred at high frequencies, with local reactions noted particularly at a high frequency upon the first administration, but no serious adverse reactions occurred. Considering that the target disease of the H5N1 Vaccine is extremely serious, PMDA concluded that no particular problems are noted with respect to its tolerability.

PMDA considers it necessary to collect detailed post-marketing information on the immunogenicity and safety of the H5N1 Vaccine in children, and in particular to assess the safety as soon as possible. In addition, the immunogenicity and safety in elderly people as well as the efficacy (immunogenicity) and safety of the H5N1 Vaccine manufactured with a different vaccine strain in place of strain NIBRG-14 should be confirmed. Also, it is desirable to assess the antigen reduction as well as the cross-protective effects, assuming the use of the H5N1 Vaccine in an actual pandemic.

Review Report (2)

August 15, 2007

I. Summary of the product

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “HOKKEN” (Proposed name: Adsorbed Influenza Vaccine “HOKKEN”)
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Kitasato Institute
[Date of application]	January 30, 2007

II. Contents of Review

The Pharmaceuticals and Medical Devices Agency (hereinafter referred to as PMDA) discusses below the matters related to quality that had not been reviewed at the time of preparation of the Review Report (1), and the matters related to the study results submitted after Expert Discussion.

Also, PMDA sought the comments of the Expert Advisors on the Review Report (1). A summary of the review based on the discussion of the Expert Advisors and also a summary of the review of the data submitted after the Expert Discussion are described below.

The Expert Advisors participating in the Expert Discussion have remarked that Item 1 and Item 2 (1) of “Immediate measures for the issue of conflict of interest involving outside experts in PMDA,” dated May 8, 2007, is not applicable to the H5N1 Vaccine.

1. Quality

The issues for which the review was not completed by the time of preparation of the Review Report (1) and the test results submitted after the Expert Discussion are as follows.

(1) Impact associated with changes in the manufacturing method

The manufacturing method of the H5N1 Vaccine was changed from non-addition to addition of the formalin stabilizer to the bulk in the manufacturing process after submission of the application for approval; the applicant compared actual measurements obtained from the specification testing of 3 lots each from bulks manufactured with and without the stabilizer and drug products from the bulks thus obtained, and presented the report stating that there are no specific changes in quality, irrespective of the presence or absence of the stabilizer [see “Review Report (1), 2. Data relating to quality, *Summary of the submitted data*, (1) Bulk, 1) Manufacturing method, e. History of development of the manufacturing process”]. The applicant subsequently submitted the results of the pharmacology study (immunogenicity test) and gave the following explanation.

Comparison of the serum antibody titers on Day 14 in mice inoculated with the drug products from bulks manufactured with and without the stabilizer revealed similar changes in HI antibody and neutralizing antibody titers in the two drug products. Therefore, the applicant considers the immunogenicity (pharmacological effect) of the two products to be equivalent, irrespective of the presence or absence of the stabilizer. In addition, no differences were observed in general conditions of the animals, including changes in body weight, between the two products.

Furthermore, the applicant stated that both drug products conformed to the acceptance criteria for the abnormal toxicity test and the pyrogen test, and that pyrogenicity was lower for the drug product from the bulk manufactured with the addition of the stabilizer than that from the bulk manufactured without it [see “Review Report (1), 2. Data relating to quality, *Outline of Review by PMDA*, (3) Characterization of bulk”]; thus, the applicant considers that the addition of the stabilizer to the bulk poses no problems in terms of safety.

PMDA accepted the above description, taking into consideration the results of the stability testing, as described below.

(2) Stability testing

The applicant submitted the results of 3-month stability testing of the bulk manufactured with the addition of the stabilizer and the drug product from the bulk thus obtained, and provided the following explanation. Long-term testing was conducted to determine the protein content and HA content (by SRD) for the bulk; and the protein content (entire protein, precipitation), potency (by SRD), and pH for the drug product. The results revealed that there were no changes in any of the above parameters 3 months after the beginning of stability testing. In the accelerated testing of the bulk, the results of the protein content, HA content, pH, and characterization (electron-microscopic observation and sucrose density-gradient centrifugation) were obtained at Months 0, 1, and 3; and they did not show any significant changes following the storage relative to baseline.

At the same time, both the long-term testing and the accelerated testing were conducted for the bulk manufactured without adding the stabilizer and the drug product from the bulk thus obtained. Based on the stability testing results, PMDA confirmed that no specific changes have been observed so far in the stability of both the bulk and the drug product, irrespective of the presence or absence of the stabilizer.

PMDA asked the applicant to further evaluate the stability, including the high-order structure. The applicant responded that based on the results obtained from further evaluation, the extension of storage time shall be filed for approval for a partial change.

(3) Reference standard

PMDA asked the applicant the reason for changing the storage temperature of the standard influenza HA

antigen (for SRD) used to determine the HA content (by SRD) of the bulk and the potency (by SRD) of the drug product from $\blacksquare \pm \blacksquare^{\circ}\text{C}$ to $\blacksquare \pm \blacksquare^{\circ}\text{C}$ for 1 lot, and from $\blacksquare \pm \blacksquare^{\circ}\text{C}$ to $\blacksquare \pm \blacksquare^{\circ}\text{C}$ for the subsequent lot. The applicant responded as follows.

The reference standard had been stored frozen at $\blacksquare \pm \blacksquare^{\circ}\text{C}$, but the storage condition was subsequently changed to $\blacksquare \pm \blacksquare^{\circ}\text{C}$, according to the information that the National Institute of Infectious Diseases, by which the reference standard was controlled and supplied, applies storage condition under refrigeration ($\blacksquare \pm \blacksquare^{\circ}\text{C}$). Thereafter, the National Institute of Infectious Diseases instructed the applicant to store the reference standard under freezing, because the potency may decrease when stored under refrigeration. For the next lot, the reference standard was stored at $\blacksquare \pm \blacksquare^{\circ}\text{C}$ for 6 months, and thereafter, stored at $\blacksquare \pm \blacksquare^{\circ}\text{C}$. Regarding the reference standard used in the specification testing and the stability testing, the former lots (i.e., first stored frozen, then under refrigeration) were used for the bulk manufactured without adding a stabilizer and the drug product from the bulk thus obtained; and the latter lots (i.e., first stored under refrigeration, then stored frozen) were used for the bulk manufactured with the addition of the stabilizer and the drug product from the bulk thus obtained. Because the former reference standard lots had been stored frozen until about 10 days before the measurement of samples stored for 6 months in the stability testing, as described in the application document, it is less likely that the potency of the reference standard may have decreased by that time. Also, no changes in the ring diameter were observed in the SRD test of the relevant lot reference standard 1 year after the beginning of the stability testing. Thus, based on the stability testing results submitted at the application, it can be concluded that the storage period be set as 6 months.

PMDA understood the applicant's explanation about the lack of impact of the storage temperature on the stability testing results at 6 months. Regarding the latter reference standard lots which are used for the currently ongoing stability testing of the bulk and the drug product, however, PMDA asked the applicant the reason for using 5 pooled bottles of the reference standard for 1 test. The applicant provided the following explanation. The latter reference standard lots were found to show large variations in the protein content and weight per vial (CV = $\blacksquare\%$; protein content, $\blacksquare\%$; weight). However, because newly prepared reference standard lots were not available, the reference standard was to be temporarily used by pooling \blacksquare bottles. Currently, a new reference standard is being prepared.

There was not enough time to establish a quality control system for the reference standard, including the stability evaluation, optimization of the storage condition, etc., because development of the H5N1 Vaccine was urgently sought. In view of this background, PMDA asked the National Institute of Infectious Diseases through the Ministry of Health, Labour and Welfare to establish a quality control system for the reference standard as soon as possible. In addition, PMDA requested the applicant to take measures such as the review of acceptance criteria, as required, through the re-evaluation of the stability and the determination of HA content and potency of the product by the SRD method at the time when the quality of the reference standard is fully ensured. The applicant replied that appropriate measures, as

stated above, would be taken.

(4) Specifications and test methods of the bulk

PMDA asked the applicant as to whether ovalbumin content, which is recommended by the WHO guideline (WHO TRSNo.927, 2005) for the inactivated influenza vaccine, should be included in the specifications because the H5N1 Vaccine is purified from embryonated eggs; in response, the applicant added this parameter to the specifications, and PMDA accepted this, including the acceptance criteria.

PMDA requested the applicant to review the following parameters included in the specifications for the bulk: pH, bacterial endotoxins, thimerosal content, formaldehyde content, protein content, HA content, and pyrogen, because the rationale for setting their acceptance criteria has not yet been defined. In response, the applicant made the appropriate corrections based on the actual values. As stated in “(3) Reference Standard,” the acceptance criteria for the HA content are to be reviewed at the time when the quality of the reference standard is established.

PMDA accepted the above.

When SRD reagents for are not available, the HA content determination is to be performed as a specification testing for the bulk; the HA content is calculated based on the result of the percent HA content determination performed for the intermediate purified virus suspension prior to inactivation. The value calculated from the testing for determining the percent HA content was quite high as compared with those measured for the same sample at the National Institute of Infectious Diseases and Saikin Seizai Kyokai. Therefore, PMDA requested the applicant to check whether there is any problem with the measurement method; in response, the applicant revised the analytical method under the direction of National Institute of Infectious Diseases and corrected the result of the HA content determination for the lot analysis, as shown in the application dossier, by re-measurement. The percent recovery, as described in “Review Report (1), 2. Data relating to quality, *Outline of the review by PMDA*, (1) Manufacturing process control of the bulk, 2) Purification process,” was corrected based on the measurement value after modifying the analytical method, and the percent HA content and the recovery of HA content per egg decreased by about 20%. PMDA accepted the above, based on the premise that the analytical validation of the relevant method could be ascertained by the time of approval.

(5) Specifications and test methods of the drug product

PMDA requested the applicant to include content uniformity test in the specifications for the drug product because the H5N1 Vaccine is an injectable suspension. Furthermore, it also requested the applicant to determine whether labeling should be included in the specifications because no test is available to identify the active ingredient contained in the drug product in the circumstance where the potency is not determined by SRD because of the lack of availability of the necessary reagents in an emergency etc. The applicant thus included both the content uniformity test and labeling in the

specifications for the drug product. In addition, PMDA requested the applicant to determine whether insoluble particulate matter should be included in the specifications, because the General Rules for Preparations of 15th Edition of Japanese Pharmacopoeia proposes that injectable suspensions be subject to the insoluble particulate matter test. The applicant responded that insoluble particulate matter would be included in the specifications for the drug product after confirming the feasibility of this testing through solubilization by the time of obtainment of the approval.

PMDA also requested the applicant to review the acceptance criteria of the following parameters for the specifications of the drug product based on the measurement values: protein content, potency, pH, aluminum content, thimerosal content, and formaldehyde content. The applicant corrected the acceptance criteria for these parameters in an appropriate manner. Acceptance criteria for the potency are to be reviewed at the time when the quality of the reference standard is established, as stated in “(3) Reference Standard”. PMDA accepted the above.

(6) Specifications of excipients

PMDA requested the applicant to review parameters in the specifications for aluminum hydroxide gel; in response, the applicant added description, identity, purity, adsorption, abnormal toxicity, bacterial endotoxins, and particle size distribution to specifications. The acceptance criteria for pH were revised to “around neutral” by the addition of a cleaning process in the manufacturing process. PMDA accepted the above.

(7) Biological materials and their control

PMDA asked the applicant to explain the measures against adventitious virus contamination during the manufacturing process of the product. The applicant submitted the information on the evaluation of virus clearance and responded as follows. The virus clearance was evaluated in the inactivation process using the non-enveloped RNA virus [REDACTED] as a model virus having a strong resistance to chemical treatment, such as formalin treatment. Because the treatment period for the inactivation process was as long as 28 days at the longest, the clearance index decreased by [REDACTED] to [REDACTED] even in the absence of the formalin stabilizer. When the clearance index obtained following formalin treatment was deducted from that in the absence of formalin, it was [REDACTED] to [REDACTED]. These values may not ensure complete virus clearance. However, since many of the viruses having a high potential for contaminating eggs are enveloped viruses, it is considered feasible to inactivate most adventitious viruses to the same degree as the influenza virus. The clearance index of the NIBRG-14 strain influenza virus was [REDACTED] in the inactivation process. Thus, the adventitious viruses are also anticipated to decrease to the same degree as the influenza virus.

PMDA considers as follows. Virus validation studies in the inactivation process were limited to [REDACTED] and the clearance index is not considered to be satisfactory. Also, it remains to be identified whether other viruses possibly having a higher sensitivity to inactivation treatment than

██████████ have been appropriately removed. PMDA requested the applicant to check for the clearance in the inactivation process of chicken leukemia virus (enveloped), chicken adenovirus (non-enveloped), etc., which are recommended by the WHO (WHO TRS No.927, 2005) as model viruses for the evaluation of the inactivation process of egg-derived inactivated influenza vaccine. In addition, PMDA proposed that the virus-free test be performed for intermediates (bulk, etc.) until the results from virus validation studies are shown. Furthermore, PMDA proposed that mycoplasma contamination be controlled, because mycoplasma control is conducted on a level of virus seed, but neither control measures nor clearance evaluation of mycoplasma has not yet been undertaken for any other raw materials or intermediates. Although adult chickens are controlled by vaccination and the like, the actual control system for embryonated eggs is not considered to be adequate because eggs hatched by adult chickens whose antibody titers after inoculation deviated from the acceptance criteria may be used. Therefore, PMDA requested the applicant to review the control system.

The applicant responded as follows. The control system for adult chickens will be reviewed in an appropriate manner, for example, by improving the control at the step of the antibody titer tests after vaccination. To establish the clearance of infectious substances in the inactivation process, validation of the inactivation will be conducted by around █████, 20██ by using Newcastle disease virus (enveloped), which has the potential to infect humans, and by around █████, 20██ by using chicken leukemia virus and chicken adenovirus. Also, clearance evaluation of mycoplasma will be conducted by █████, 20██. The applicant will submit the results when they are available. For the bulk, the applicant commits to continue the adventitious virus testing and mycoplasma testing until the relevant study results show the safety of the bulk in terms of adventitious infectious substances. The detection sensitivity of virus-free testing will be confirmed by around █████, 20██ by using viruses with a potential of contamination via egg transmission.

PMDA accepted the above.

(8) Others

The issues for which the applicant has committed to confirm additionally at the time of preparation of Review Report, (1) are described below.

· [(2) Manufacturing process control of the bulk 1) Inactivation process]

The applicant submitted additional validation data of the specificity of the inactivation test and explained as follows. The maximum number of subcultures in eggs that allowed the hemagglutination (HA titer) was determined for the inactivated NIBRG-14 strain and Indonesian strain. For the NIBRG-14 strain, when the protein concentration of the sample was in the range of █████-█████ μg/mL, HA titer was detected in the █th subculture, but below the detection limit after the █th subculture. For the Indonesian strain, on the other hand, when the protein concentration of the sample was in the range of █████-█████ μg/mL, HA titer was detected in up to the █th subculture, but completely below the detection limit in the █th

subculture even with high protein concentration. These results proved that HA titer of virus derived from the inoculated material is no longer detected after the ■th subculture, although HA titer of some virus strains may be detected in up to the ■th subculture, even if samples are no more infectious. In view of the fact that infectious viruses have been propagated in the ■th subculture, the present inactivation testing has the ability to detect any residual infectious viruses specifically.

PMDA accepted the above explanation.

· **[(4) Manufacturing process control of the drug product]**

The applicant conducted further validation studies of the homogeneity of the filling. A mock-up vaccine was investigated on an actual manufacturing level using aluminum hydroxide gel alone and the actual vaccine solution was investigated on a 1/■ pilot scale. These results provided evidence showing that even the present suspension product is filled homogeneously. PMDA accepted the above.

· **[(5) Analytical validation of the test methods]**

The results of the analytical validation of the tests were submitted additionally. The applicant explained the adequacy of the tests by showing the results of additional investigations of the specificity, intermediate precision, detection limit etc., including the above inactivation test, and the validation results will be submitted before approval is obtained for the percent HA content measurement (in-process control test) for which the analysis method was changed.

PMDA accepted the above.

2. Toxicology studies

The study of the effect of the H5N1 Vaccine on pre- and postnatal development of offspring and maternal function, which was reported as ongoing at the time of preparation of the Review Report (1), was completed and the data were submitted. The H5N1 Vaccine was injected subcutaneously at doses of 0.25 and 0.5 mL/kg, corresponding to 25 and 50 times the clinical dose, a total of 7 times, namely, 3 times on Days 7, 12, and 17 of gestation and 4 times on Days 0, 7, 14, and 21 after delivery. No changes attributable to the H5N1 Vaccine were observed in either the general condition, etc. of the dams or the development, etc. of the F1 offspring. From these results, NOAEL was estimated to be 0.5 mL/kg or above, both for the dams and for the F1 offspring.

PMDA concluded there is no major problem with the toxicology study data, including the above study results that were submitted after the application, taking into account the matters discussed at the Expert Discussion.

3. Efficacy

Although HI antibody titer data obtained with chicken erythrocytes failed to suggest a protective effect of administration of the H5N1 Vaccine against pandemic influenza virus infection, (a) neutralizing antibody titer data demonstrated induction of antibody production by administration of the H5N1 Vaccine and (b)

a rise in HI antibody titer was achieved in a substantial proportion of the clinical study subjects despite a reportedly low sensitivity of HI antibody titer assay when applied to the H5 antigen. PMDA has therefore concluded that administration of the H5N1 Vaccine has the potential to result in acquisition of immunity against pandemic influenza, thereby reducing clinical symptoms and decreasing fatalities associated with this disease.

The Expert Advisors made the following comments.

Although the efficacy of a vaccine should generally be demonstrated by confirmation of its protective effect against infection, it is difficult to assess protection against pandemic influenza virus infection in advance. Furthermore, associations between HI antibody titer or neutralizing antibody titer and protection against infection are not sufficiently clear for pandemic influenza, which is a limitation in the efficacy assessment of the H5N1 Vaccine on the basis of these antibody titers. Nevertheless, the efficacy of the H5N1 Vaccine must be assessed in terms of immunogenicity because no other indices are as yet available. Considering that a similar inactivated whole virus influenza vaccine showed a protective effect against infection in mice, the conclusion by PMDA that a protective effect of the H5N1 Vaccine against virus infection in humans may well be expected to be reasonable. Thus, the conclusion was supported by the Expert Advisors. Methods for antibody titer assay were also discussed. PMDA considers it difficult to compare the immunogenicity of the H5N1 Vaccine with those of similar drug products available in Japan and overseas due to the absence of a domestically standardized method (i.e., standardized among the vaccine manufacturing sites in Japan) or an internationally standardized method. This was accepted by the Expert Advisors.

The Expert Advisors also made the following comment: Long-term changes in post-vaccination antibody titer should be confirmed by follow-up assessment. Since follow-up assessment of antibody titer had been scheduled 90 days and 180 days after administration of the H5N1 Vaccine based on discussion at clinical trial consultation during its development, PMDA asked the applicant to immediately present the available follow-up data.

The applicant responded as follows.

A clinical study was conducted in the subjects of Study KIB-PIA02 to measure HI antibody titer (equine erythrocytes) and neutralizing antibody titer 90 days and 180 days after the first administration of the investigational vaccine. Although the final follow-up report according to the statistical analysis plan of this study will be completed by ■■■ to ■■■■■, the following limited data is currently available: although HI antibody titer (equine erythrocytes) did not tend to decrease 90 days and 180 days after the first vaccination as compared with the level at the post-study investigation for Study KIB-PIA02, neutralizing antibody titer did. In particular, the value at 180 days after the first vaccination decreased to a level comparable to that determined prior to the second administration in Study KIB-PIA02.

Changes in antibody titer

Assessment timing	Dose group	Number of subjects*	Neutralizing antibody titer (fold)		HI antibody titer (fold)	
			Geometric mean	Standard deviation	Geometric mean	Standard deviation
Prior to first administration	5 µg	150	5.8	1.33	5.1	1.29
	15 µg	150	5.9	1.34	5.3	1.38
Prior to second administration	5 µg	150	12.3	1.77	6.5	1.74
	15 µg	150	14.5	1.89	7.4	1.92
Post-study investigation	5 µg	149	21.3	1.97	8.8	2.14
	15 µg	149	29.8	2.04	13.5	2.27
90 days after first administration	5 µg	140	19.4	2.26	11.8	1.50
	15 µg	146	24.4	2.31	13.3	1.66
180 days after first administration	5 µg	130	13.8	1.66	13.0	1.67
	15 µg	129	17.3	1.77	15.6	1.89

* Antibody titer data prior to first and second administrations, and on post-study investigation, were obtained in the FAS in Study KIB-PIA02, while follow-up data were obtained 90 days and 180 days after the first administration in the subjects of Study KIB-PIA02 who gave informed consent to participate in the follow-up study.

Based on the data presented above, the applicant explained the considerations in use of the H5N1 Vaccine as either a pre-pandemic or a pandemic vaccine as follows:

When used as a pre-pandemic vaccine, an additional administration of the H5N1 Vaccine 180 days after the first administration is desirable to assure a protective effect against infection, because the antibody titer at this time point decreased to a level comparable to that determined prior to the second vaccination (3 weeks after the first administration). An additional administration 180 days after the first administration is also desirable when it is used as a pandemic vaccine. However, because the finding that an antibody titer comparable to that determined prior to the second vaccination (3 weeks after the first administration) was maintained even 180 days after the first administration suggests that the protective effect of the H5N1 Vaccine against infection primed by the first administration may persist at least to some extent at this time point, and also because vaccination of an increased number of recipients is expected to exhibit a greater protective effect against infection at the population level, the H5N1 Vaccine-naïve individuals should preferentially be vaccinated when the vaccine supply is limited.

PMDA considers as follows.

The Pandemic Influenza Preparedness Action Plan of the Japanese Government and Guidelines on Vaccination against Pandemic Influenza state that, at WHO Phase 4 when human-to-human transmission of a pandemic influenza virus is confirmed, the following countermeasures will be implemented: commencement of vaccination with pre-pandemic vaccines, acquisition of a pandemic influenza virus strain for vaccine production immediately followed by commencement of manufacturing of pandemic vaccines, and commencement of vaccination with pandemic vaccines. Neutralizing antibody titer tended to decrease over time after vaccination, as already stated. Therefore, it is necessary to investigate a need for re-vaccination after a prolonged interval from the initial vaccination (e.g., an interval from the initial

vaccination with a pre-pandemic or pandemic vaccine to the first wave of a pandemic at WHO Phase 6 or an interval from vaccination with a pandemic vaccine during or after the first wave of a pandemic to a subsequent second or third wave of the identical pandemic) based on the completed final report of the aforementioned clinical study, and to implement an additional clinical study to confirm the efficacy and safety of booster vaccination (re-vaccination) after a certain period following the initial vaccination.

4. Safety

Although adverse reactions occurred at high frequencies with injection site reactions noted particularly at a high frequency upon the first administration, such frequent development of intense local (injection site) reactions can be readily predicted because the H5N1 Vaccine is not a split vaccine (a vaccine consisting of a mix of virus components but retaining no virion structure) like seasonal influenza vaccines but does contain inactivated whole viruses with a potentially higher immunogenicity as the active ingredient as well as aluminium hydroxide as an adjuvant. On the other hand, no serious adverse reactions occurred except those at the injection site. Considering that the target disease of the H5N1 Vaccine is extremely serious, PMDA concluded that there are no particular problems with respect to its tolerability based on the submitted safety data.

The above PMDA conclusion was supported by the Expert Advisors. Moreover, the Expert Advisors made the following comments.

Although adverse reactions associated with the H5N1 Vaccine are tolerable, their frequencies are higher than those of other vaccines previously approved in Japan. Therefore, safety information such as the type and frequency of individual adverse reactions reported in the clinical studies should be provided to the recipients of the H5N1 Vaccine. Also, information on risks and benefits associated with vaccination with the H5N1 Vaccine should be fully provided prior to actual vaccination, because to what extent the immunity against pandemic influenza acquired by administration of the H5N1 Vaccine is actually effective in preventing infection has not yet been confirmed. Moreover, the Expert Advisors stated: Quick and detailed safety data collection is crucial, because the H5N1 Vaccine will be administered to an extremely large number of recipients within a short time when actually used [see “Review Report (1), 4. Clinical data, *Outline of review by PMDA*, (6) Post-marketing considerations”].

5. Clinical positioning and indications

Based on the results of assessment of the immunogenicity and safety of the H5N1 Vaccine, a mock-up vaccine produced with influenza virus strain NIBRG-14, PMDA has concluded that it has the potential to be useful as a vaccine against H5N1 influenza. Furthermore, considering that a similar inactivated whole virus influenza vaccine showed a protective effect against infection in mice and based on historical experiences of previous influenza pandemics such as the “Spanish flu,” “Asian flu,” and “Hong Kong flu” [see “Review Report (1), 4. Clinical data, *Outline of review by PMDA*, (3) Clinical positioning”], PMDA considers the H5N1 Vaccine to potentially exhibit a protective effect against pandemic influenza

or prevent symptoms becoming more severe and concluded that the H5N1 Vaccine should be positioned as a prophylactic vaccine against pandemic influenza virus infection. Thus, PMDA has concluded that it is acceptable to state in the INDICATIONS section that the H5N1 Vaccine is indicated for “prophylaxis of pandemic influenza.”

Although the Expert Advisors supported the above PMDA conclusion, they stated that the subtype of pandemic influenza as the target of the H5N1 Vaccine should be defined more clearly in the INDICATIONS section.

In the actual production of a pandemic influenza vaccine according to the procedure described for the H5N1 Vaccine, the Japanese Ministry of Health, Labour and Welfare and relevant authorities are expected to cooperate with reference laboratories of WHO, World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) for identification and analysis of a pandemic influenza virus strain and to develop attenuated candidate virus strains for production of vaccines. The applicant will then produce the vaccine using a vaccine strain designated by the Ministry of Health, Labour and Welfare. Therefore, there may actually be no trouble if the subtype of the pandemic influenza virus used for vaccine production is not specified in the INDICATIONS section. On the other hand, it is impossible at present to predict the seriousness of the disease associated with infection with a pandemic influenza virus belonging to a subtype other than H5N1, making assessment of the risk-benefit balance difficult. Therefore, the Expert Advisors stated that the product should be indicated exclusively for influenza of subtype H5N1.

PMDA communicated these comments to the applicant and the applicant responded that the statement in the INDICATIONS section will be changed as follows: the product is indicated for “prophylaxis of pandemic influenza (H5N1).”

PMDA accepted the applicant’s response.

6. Dosage and administration

Study KIB-PIA02 involved intramuscular administration exclusively, because Study KIB-PIA01 demonstrated that the frequency of adverse reactions tended to be lower in the intramuscular administration group than the subcutaneous administration group and showed the possibility that a greater rise in antibody titer might be obtained in the intramuscular administration group. Study KIB-PIA01 demonstrated that no serious adverse events occurred or neither the second administration of the H5N1 Vaccine nor the clinical study was discontinued because of adverse events in the subcutaneous administration group and that Grade 3 or Grade C or higher adverse events eventually resolved. PMDA has therefore concluded that there is no need or reason for rejecting subcutaneous injection, considering that vaccinations in Japan basically involve subcutaneous injection. In addition, because a rise in antibody titer to the extent possible is desirable, with the threshold antibody titer in relation to a

protective effect against infection being unknown, PMDA has concluded that the DOSAGE AND ADMINISTRATION should be “The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses,” with the antigen content per dose (0.5 mL) specified as “15 µg (in terms of HA antigen)” in the columns of Ingredients and their quantity and Manufacturing method in the approval certificate.

Although the Expert Advisors supported the above PMDA conclusion, they stated that descriptions in the DOSAGE AND ADMINISTRATION section of the package insert should be improved so that information specific to each route of administration, including efficacy and safety information, is provided unambiguously to on-site medical personnel.

PMDA requested the applicant to modify descriptions in the DOSAGE AND ADMINISTRATION section of the package insert, taking the above comment into account. The applicant handled them appropriately.

7. Post-marketing considerations

PMDA considered it necessary to collect post-marketing information on the following topics in order to further assess populations not included in the clinical studies and the usefulness of the H5N1 Vaccine as a countermeasure for pandemic influenza.

- a. Children
- b. Elderly people
- c. Efficacy and safety of the H5N1 Vaccine manufactured with a vaccine strain other than NIBRG-14
- d. Cross-protective effects
- e. Antigen saving

The Expert Advisors made the following comments: Whether information collection on these 5 topics is possible as a part of post-marketing surveillance of the H5N1 Vaccine used according to the Pandemic Influenza Preparedness Action Plan and whether additional clinical studies are necessary should be assessed as soon as possible; in particular, prompt information collection on the use of the H5N1 Vaccine in children and elderly people, populations experiencing particularly high fatalities, is urgently needed.

PMDA communicated these comments to the applicant, and the applicant responded as follows.

- a. Since there is concern that influenza of subtype H5N1 may be associated with a high infectivity (incidence) and fatality rate in children, a clinical study should be conducted to evaluate the immunogenicity and safety of the H5N1 Vaccine in children. When such a clinical study is actually implemented, subject stratification by age and the vaccine dose employed must be investigated in detail, considering information on dosage and administration of current influenza HA vaccines and previously available whole virus influenza vaccines in children as well as safety information.

Therefore, the applicant will discuss concrete implementation methods of the further clinical study with relevant experts.

- b. Although a post-vaccination rise in antibody titer may be smaller in individuals aged 65 years or older than in healthy non-elderly adults, there is no safety concern to exclude vaccination with the H5N1 Vaccine in such elderly people, considering the situations associated with geriatric use of seasonal influenza vaccines. The applicant intends to collect information on immunogenicity and safety in elderly people on the occasion of vaccination with the H5N1 Vaccine as a pre-pandemic vaccine. Actually, however, recipients of pre-pandemic vaccines and the priority in vaccination among them are determined by the Ministry of Health, Labour and Welfare. Also, as a rule, vaccination is conducted as part of a mass vaccination program organized by the relevant local government at vaccination sites (e.g., local health centers) specified by the organizing government. Furthermore, collection of safety data using a health status questionnaire and post-vaccination antibody titer assay are planned for some vaccine recipients. Therefore, information collection with respect to geriatric use of the H5N1 Vaccine will be conducted in the future after discussion and assessment with relevant authorities including administrative agencies on detailed timing and procedures (e.g., information collection method).
- c. As for seasonal influenza vaccines (influenza HA vaccines), the virus strain used for vaccine production is changed every year and no major difference in safety has been noted between vaccines produced using different virus strains. Also, considering that (1) a vaccine that has any abnormality in quality testing data, such as abnormal toxicity and pyrogenicity, and does not meet the specifications is not shipped, and that (2) the immunogenicity of a vaccine is controlled by HA content assay as a part of quality control during its production process, the immunogenicity and safety of the H5N1 Vaccine produced with a different vaccine strain but according to an identical procedure may be comparable to those of the original H5N1 Vaccine. However, since no clinical study data is as yet available for the H5N1 Vaccine produced using a virus strain other than NIBRG-14, it is important to confirm the immunogenicity and safety of the H5N1 Vaccine produced with a virus strain different from NIBRG-14. However, the applicant must consult with administrative agencies on the study of the human immunogenicity and safety of a drug product when it is produced from the stock solution, which is produced for stockpiling and currently procured by the national government. In addition, the Ministry of Health, Labour and Welfare has the authority to determine the virus strain to be used for production of the stock solution for stockpiling as well as the vaccine strain to be used for pre-pandemic or pandemic vaccine production and to decide on the clinical use of the vaccine thus produced. In other words, the applicant is not authorized to determine which strain other than NIBRG-14 is to be investigated for comparison. Accordingly, how to assess the efficacy and safety of a drug product produced with a virus strain other than NIBRG-14, including the strain to be used as well as timing, will be determined in the future after discussion and assessment with relevant authorities including administrative agencies.

- d. The protocol for Study KIB-PIA02 states that the activity of cross-neutralization against H5N1 influenza virus strains, other than that used for production of the H5N1 Vaccine, is assayed at [REDACTED] as necessary, and serum specimens collected from the subjects of Study KIB-PIA01 who gave informed consent have been provided to [REDACTED]. The applicant will discuss and assess concrete procedures for assessing cross-neutralization, such as the kind of virus strain, with relevant authorities including administrative agencies.
- e. The applicant understands the importance of investigating the clinical usefulness of a drug product with reduced antigen content (low-dose injection) and considers it necessary to assess the usefulness of low-dose injection of the H5N1 Vaccine including its priming effect. However, due to limitations in the applicant's capacity to produce a pandemic influenza vaccine and procurement of the stock solution by the national government, it is difficult to separately produce a drug product with reduced antigen content and promptly start clinical studies. This issue is closely related to vaccine policy as a part of the national countermeasures for pandemic influenza and should be assessed after extensive discussion with relevant authorities including administrative agencies.

PMDA accepted the applicant's response and directed the applicant to conduct the clinical study of the H5N1 Vaccine in children as soon as possible, collect information on the clinical use of the H5N1 Vaccine in elderly recipients without delay, and actively assess detailed procedures for items c. to e. after discussion with relevant authorities. The applicant understood the above directions.

The Expert Advisors made the following comments.

Information on the efficacy and safety of the H5N1 Vaccine should be fully provided prior to vaccination, including the fact that it is unknown to what extent the immunity against pandemic influenza acquired by vaccination with the H5N1 Vaccine is actually effective in preventing infection. Also, quick and detailed safety data collection is crucial, because the H5N1 Vaccine is administered to an extremely large number of recipients within a short time when actually used. The Guidelines on Vaccination against Pandemic Influenza states that the vaccination is planned, as a rule, as a mass vaccination organized by prefectural governments (for pre-pandemic vaccines) or municipal governments (for pandemic vaccines). In conducting actual vaccination, the following are planned in accordance with the Guidelines: Currently available information is to be provided extensively to vaccine recipients by, for example, distribution of leaflets describing efficacy and adverse reactions of the vaccine and holding an explanatory meeting as necessary to educate target individuals regarding vaccination. Vaccine recipients are to be ordered to stay at the vaccination site for at least 30 minutes after injection to watch for serious adverse reactions, such as anaphylactic shock, and safety data are to be collected from some recipients using a health status questionnaire as post-vaccination surveillance, in a manner similar to a post-immunization health status survey following a routine immunization. In addition, when a vaccine recipient is aware of symptoms suspected to be adverse reactions associated with injection of a pandemic influenza vaccine or a doctor

identifies a vaccine recipient suspected to have such adverse reactions, immediate communication of this fact must be made to the prefectural or municipal government organizing mass vaccination with subsequent prompt reporting by the alerted local government to the Ministry of Health, Labour and Welfare using the National Epidemiological Surveillance of Infectious Disease (NESID) system in an emergency. PMDA considers it to be necessary that administrative agencies take the initiative on information delivery and safety data collection with respect to pandemic influenza vaccines, because they are in charge of selecting vaccination targets and the organization of mass vaccination. Based on these assumptions, PMDA requested the applicant to actively assess and discuss with administrative agencies what to do as part of the responsibilities of the marketing authorization holder of the H5N1 Vaccine when the vaccine is actually used as part of the national countermeasures against pandemic influenza. The applicant understood the above request.

On the other hand, the Expert Advisors made the following comment with respect to post-marketing surveillance of the efficacy of the H5N1 Vaccine: Although detailed future assessment is necessary on the extent to which detailed surveying is possible in situations of an actual influenza pandemic, data on the protective effect against infection should be collected to the extent possible. In fact, in cooperation with medical institutions and local governments involved in vaccination against pandemic influenza, collection of blood specimens from some of the vaccine recipients after obtaining informed consent for antibody titer assay at the National Institute of Infectious Diseases is planned according to the Guidelines on Vaccination against Pandemic Influenza. PMDA considers it desirable to assess procedures for evaluation of actual protective effects against pandemic influenza as well.

8. Brand name

The Expert Advisors made the following comments: The fact that the product is intended for emergency use exclusively upon outbreak of pandemic influenza should be clearly indicated. Based on this comment, PMDA requested the applicant to add the word that means a pandemic influenza virus strain to both the non-proprietary name and the brand name of the product. The brand name of the product was thus changed to “Adsorbed Influenza Vaccine (H5N1).”

9. Others

(1) Pre-vaccination seropositivities

The Expert Advisors stated that the clinical significance of pre-vaccination seropositive subjects observed in the clinical studies of the H5N1 Vaccine should be investigated.

PMDA asked for the applicant’s view on the meaning of pre-vaccination seropositivity against H5 antigen.

The applicant responded as follows.

It is difficult to explain the observed pre-vaccination seropositivity solely based on the data obtained in

