Report on the Deliberation Results

July 23, 2012

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

[Brand name]	Tetrabik Subcutaneous Injection Syringe
[Non-proprietary name]	Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine
[Applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	December 27, 2011

[Results of deliberation]

In the meeting held on July 20, 2012, the Second Committee on New Drugs concluded that the product may be approved and that this result should be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is classified as a biological product, the re-examination period is 8 years, and the drug substance and the drug product are both classified as powerful drugs.

Based on the data submitted by the applicant, the shelf life for the product is 18 months.

Review Report

July 12, 2012
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]	Tetrabik Subcutaneous Injection Syringe
[Non-proprietary name]	Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine
[Name of applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	December 27, 2011
[Dosage form/Strength]	A suspension for injection in 0.5-mL single-dose prefilled syringes. Each 0.5-mL dose contains ≥4 units of the *Bordetella pertussis* protective antigen, ≤15 Lf of diphtheria toxoid, ≤2.5 Lf of tetanus toxoid, 1.5 DU of inactivated poliovirus type 1 (Sabin strain), 50 DU of inactivated poliovirus type 2 (Sabin strain), and 50 DU of inactivated poliovirus type 3 (Sabin strain) as active ingredients.
[Application classification]	Prescription drug (1) Drug with a new active ingredient
[Items warranting special mention]	Expedited Review (Notification No. 1227-1 of Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated December 27, 2011)
[Reviewing office]	Office of Biologics II

Review Results

July 12, 2012

[Brand name] Tetrabik Subcutaneous Injection Syringe

[Non-proprietary name] Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine

[Name of applicant] The Research Foundation for Microbial Diseases of Osaka University

[Date of application] December 27, 2011

[Results of review]

Based on the submitted data, the efficacy of the product in preventing pertussis, diphtheria, tetanus, and acute poliomyelitis has been demonstrated and its safety is acceptable in view of its observed benefits. It is necessary to collect information on post-vaccination convulsions and febrile convulsions via post-marketing surveillance.

As a result of its review, the Pharmaceuticals and Medical Devices Agency concluded that the product may be approved for the following indication and dosage and administration.

[Indication]

Prevention of pertussis, diphtheria, tetanus, and acute poliomyelitis

[Dosage and administration]

Primary immunization: The usual primary series for children consist of three doses of 0.5 mL each given by subcutaneous injection at intervals of at least 3 weeks.

Booster immunization: The usual booster dose for children is a single 0.5 mL dose given by subcutaneous injection at least 6 months after the primary immunization.

Review Report (1)

June 8, 2012

I. Product Submitted for Registration

[Brand name] Tetrabik Subcutaneous Injection Syringe

[Non-proprietary name] Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine

[Name of applicant] The Research Foundation for Microbial Diseases of Osaka University

[Date of application] December 27, 2011

[Dosage form/Strength] A suspension for injection in 0.5-mL single-dose prefilled syringes. Each 0.5-mL dose contains ≥4 units of the *Bordetella pertussis* protective antigen, ≤15 Lf of diphtheria toxoid, ≤2.5 Lf of tetanus toxoid, 1.5 DU of inactivated poliovirus type 1 (Sabin strain), 50 DU of inactivated poliovirus type 2 (Sabin strain), and 50 DU of inactivated poliovirus type 3 (Sabin strain) as active ingredients.

[Proposed indication] The product is used for the prevention of pertussis, diphtheria, tetanus, and acute poliomyelitis.

[Proposed dosage and administration]

Primary immunization:

The usual primary series consist of three doses of 0.5 mL each given by subcutaneous injection at 3- to 8-week intervals.

Booster immunization:

The usual booster dose is a single 0.5 mL dose given by subcutaneous injection at least 6 months after the primary immunization (normally 12-18 months after the completion of primary immunization).

[Items warranting special mention]

Expedited Review (Notification No. 1227-1 of Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated December 27, 2011)

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

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

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

The product (Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio [Sabin strain] Combined Vaccine: DPT-sIPV vaccine) is a combination vaccine containing the protective antigens of *Bordetella pertussis*, diphtheria toxoid, and tetanus toxoid of Tribik® (as the "Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine" listed in the Minimum Requirements for Biological Products) manufactured by the applicant, which was approved in 2006, and inactivated Sabin strains of attenuated poliovirus types 1, 2, and 3 that were developed by the Japan Poliomyelitis Research Institute. Each 0.5-mL dose of the product contains ≥4 units of the *Bordetella pertussis* protective antigen, ≤15 Lf of diphtheria toxoid, and ≤2.5 Lf of tetanus toxoid and 1.5 D-antigen units (DU) of inactivated poliovirus type 1 (Sabin strain), 50 DU of inactivated poliovirus type 2 (Sabin strain), and 50 DU of inactivated poliovirus type 3 (Sabin strain) as active ingredients and aluminum hydroxide and aluminum [redacted] as adjuvants.

Immunization against pertussis, diphtheria, and tetanus began in the US in the 1940s, with a killed whole-cell pertussis vaccine, a diphtheria toxoid vaccine, and a tetanus toxoid vaccine, respectively. A combined pertussis, diphtheria, and tetanus vaccine was introduced globally in the late 1960s and to Japan in 1968 as well. Since killed whole cells of pertussis bacteria were associated with severe adverse reactions such as post-vaccination local reactions and fever, and considered to cause serious adverse reactions such as encephalopathy, adsorbed diphtheria-purified pertussis-tetanus combined vaccines containing protective antigens purified from *Bordetella pertussis* (DPT) were developed (National Institute of Health Research Associate ed. *Vaccine Handbook.* 1994: 59-70). In Japan, the DPT developed by the applicant etc. was introduced in 1981. According to the Infectious Disease Surveillance Center, the National Institute of Infectious Diseases, since the introduction of DPT, there have been fewer incidences of epidemics of whooping cough among infants (*IASR*. 2008;29:65-66.) and diphtheria and tetanus cases have become very rare (*IASR*. 2006;27:331-332, *IASR*. 2009;30:65-66).

In Japan, epidemics of acute flaccid paralysis due to poliovirus infection, i.e. acute poliomyelitis (polio) almost ceased by the mid-1960s following the introduction of a Live Oral Poliomyelitis

Vaccine derived from attenuated strains of poliovirus (OPV), and the last reported case of polio acquired from a wild-type poliovirus was in 1980 (*Report on National Epidemiological Surveillance of Vaccine-Preventable Diseases*, 2008, Poliomyelitis: 8-15, 2011). As OPV is a live vaccine, it is known that attenuated strains of poliovirus in OPV very rarely revert to virulence and cause vaccine-associated paralytic poliomyelitis (VAPP) (*Annu Rev Microbiol*. 2005;59:587-635). According to the summary of vaccine adverse event reports (April 1, 2010 to March 31, 2011 [Committee for investigation of vaccine adverse events/heath status, Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, MHLW]), there were 38 cases of VAPP including 1 case of secondary infection from an OPV recipient in Japan between October 1, 1994 and March 31, 2011. VAPP caused by secondary infection has been reported also by 1 OPV-unvaccinated child (*J Jpn Pediatr Soc*.2011;115:800-803.) and 2 adults who were infected within their families (*Intern Med*. 2006;45:373-375, *Jpn J Infect Dis*,. 2006;59:277). In countries/regions where polio caused by wild-type viruses has been rare, inactivated poliovirus vaccines (IPV) have been introduced in order to avoid VAPP associated with OPV. As of February 2011, more than 40 countries including the US, Europe, Canada, and Korea use IPVs only. Also in Japan, the following recommendation was issued: "Early introduction of IPV is essential to stop VAPP associated with continued use of OPV" (March 31, 2003, Subcommittee on Polio and Measles Vaccines, Infection Committee, Infection Department, the 7th Health Sciences Council [hereinafter "2003 Subcommittee"]). Efforts were made towards the development/introduction of IPV in Japan, and an IPV, Imovax Polio™ for subcutaneous injection (Sanofi Pasteur) was approved on April 27, 2012.

Unlike IPV derived from virulent strains of poliovirus which are widely used in Imovax Polio™ subcutaneous etc., the inactivated poliovirus component of the product is derived from attenuated strains (Sabin strains) of poliovirus which are used in OPV, and such IPV derived from attenuated strains is globally unprecedented.

Along with regulatory submission for the product, MHLW issued a notification of the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW (PFSB/ELD Notification No. 1227-1 dated December 27, 2011 "Expedited Review and Inspection for Drug") to request PMDA to conduct expedited review and inspection.

2. Data relating to quality

2.A Summary of the submitted data

The DPT-sIPV vaccine is a combination vaccine containing the protective antigens of

Bordetella pertussis, diphtheria toxoid, and tetanus toxoid, which are the same as those used in the approved Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine, and inactivated polioviruses types 1, 2, and 3 (the antigens prepared from poliovirus [Sabin stains] particles grown in Vero cells, purified, and inactivated with formaldehyde solution) (inactivated poliovirus) as active ingredients and aluminum [redacted] and aluminum hydroxide as adjuvants.

2.A.(1) Drug substance

The drug substance consists of the bulk of purified pertussis vaccine, bulk diphtheria toxoid, bulk tetanus toxoid, and monovalent bulks of inactivated poliovirus types 1, 2, and 3 (monovalent bulks).

The Japan Poliomyelitis Research Institute submitted master files (MFs) for monovalent bulks of types 1, 2, and 3 (MF registration numbers, 221MF10287, 221MF10288, and 221MF10289) and a MF for trivalent bulk of inactivated polio vaccine comprising a mixture of monovalent bulks of types 1, 2, and 3 (trivalent bulk; MF registration number, 222MF10002).

The summary of the submitted data and the outline of the review by PMDA regarding monovalent bulks of types 1, 2, and 3 and trivalent bulk are as shown in Appendix 1. The data on the three bulks, i.e. the bulk of purified pertussis vaccine, bulk diphtheria toxoid, and bulk tetanus toxoid are summarized below.

2.A.(2) Pertussis bulk (Bulk of purified pertussis vaccine)

The pertussis bulk is a purified antigen solution containing formaldehyde-detoxified pertussis toxin (PT) and filamentous hemagglutinin (FHA) as the major protective antigens.

2.A.(2).1) Manufacturing process

(a) Preparation and control of seeds

Tohama phase I strain of *Bordetella pertussis* distributed by the National Institute of Infectious Diseases (NIID) was passaged [redacted] times, divided into small portions, and then freeze-dried to establish a master seed (MS) in 19[redacted]. The MS was passaged [redacted] times to prepare a working seed (WS). The MS and WS conformed to the tests listed in Table 2-1 and the seeds were qualified.

Table 2-1. Control tests on MS and WS of *Bordetella pertussis*

Test	MS[a)]	WS
Viable count assay	○	○
Agglutination test (agglutination reaction with anti-*Bordetella pertussis* antibody)	○	○
Microscopy (Gram staining)	○	○
Test for presence of contaminating bacteria (contaminating bacterial growth in [redacted] medium and [redacted] medium supplemented with [redacted])	○	○
Culture assay (growth of the cultures as measured by OD_{650})	○	○
Antigen production assay (hemagglutinating activities of antigens [FHA and PT])	○	○
Antigen production rate (ELISA)	○	○
Nucleotide sequencing of the antigen genes	○	○

○: Tested, －: Not tested

a) Sterility test (test for presence of contaminating bacteria using selective media etc.) was also performed when the MS was established in 19[redacted].

The MS has been stored at [redacted]°C and the WS has been stored at ≤[redacted]°C and the stability of the WS during storage will be assessed by periodically performing the tests listed in Table 2-1 excluding culture assay. In addition, viable count assay will be performed at time of use of the MS or WS. When the number of remaining ampoules of the MS or WS is decreased to a certain level, a new MS will be prepared from the MS established in 19[redacted] and a new WS will be prepared from the new MS. The newly prepared MS or WS will be qualified by the tests listed in Table 2-1.

(b) Manufacturing process and critical steps/critical intermediates and process validation

The manufacturing process for the bulk of purified pertussis vaccine is as shown in Table 2-2.

Table 2-2. Summary of manufacturing process and controls for bulk of purified pertussis vaccine

	Manufacturing process	Intermediate/Final	In-process testing
Cultivation	Seed culture (Seed preparation 1 to Seed culture 4) Inoculation of [redacted] platinum loop of WS ↓ [redacted] mL, [redacted]±[redacted]°C, [redacted] hours, under static condition ↓ [redacted]–[redacted] mL, [redacted]±[redacted]°C, [redacted] hours, under static condition ↓ [redacted]–[redacted] L, [redacted]±[redacted]°C, [redacted] hours, shaking ↓ [redacted] L, [redacted]±[redacted]°C, [redacted] hours, agitation		
	Production culture (main culture) [redacted]–[redacted] L, [redacted]±[redacted]°C, [redacted]–[redacted] hours, agitation	Cultures	Microscopy (Gram staining)
Crude purification[a)]	Clarification-1 (compression filtration by [redacted])	Clarification-1 solution	
	Concentration	Concentrate	
	Clarification-2 (compression filtration by [redacted])	Clarification-2 solution	
	Dialysis	Dialyzed solution	
	Filtration (pore size ≤[redacted]µm)	Filtrate	
	Adsorption and desorption/Salt precipitation		
	Low-speed centrifugation		
	Supernatant after centrifugation (Process flow I: [redacted] fraction)	[redacted] I	
	Pellet after centrifugation (Process flow II: [redacted] fraction) Washing of pellet /Extraction	[redacted] I (Process flow I)	[redacted] II
	Salt precipitation Salt precipitation		[redacted] II (Process flow II)
	Removal of supernatant/recovery of pellet /dissolution	Supernatant after low-speed centrifugation (Process flow I, Process flow II)	
High purification[a)]	Zonal ultracentrifugation	Supernatant after ultracentrifugation (Process flow I, Process flow II)	
	Salt precipitation	Solution after salt precipitation (Process flow I, Process flow II)	
	Dialysis	[redacted] (Process flow I, Process flow II)	
	Centrifugation	Supernatant after dialysis and centrifugation (Process flow I, Process flow II)	
	Sterile filtration (pore size [redacted]µm)	Highly purified bulk (Process flow I, Process flow II)	Endotoxins Purity of FHA[b)] (FHA-HA) Purity of PT[b)] (PT-HA)
Detoxification	Blending and dilution of bulks (FHA/PT ratio: [redacted], Protein nitrogen content, [redacted]µg/mL)	Bulk before detoxification	Protein nitrogen content Acidic PAGE (FHA/PT ratio)
	Detoxification: [redacted]–[redacted]v/w % formalin, [redacted]±[redacted]°C, [redacted]–[redacted] days	Detoxified bulk	
	Dialysis	Dialyzed detoxified bulk	
	Sterile filtration (pore size [redacted]µm)	Pertussis bulk (bulk of purified pertussis vaccine)	Filter integrity

: Critical steps or critical intermediates

a) Process flow I and Process flow II are purified separately.

b) Hemagglutinating activity of FHA or PT per µg protein nitrogen (HA titer)

The pertussis bulk or the intermediates in the manufacturing process for the pertussis bulk were evaluated for the parameters listed in Table 2-3, which demonstrated that each process step is adequately controlled to ensure consistent production.

Table 2-3. Process validation/evaluation of manufacturing process for pertussis bulk

Process step	Parameter
Cultivation[a)]	Cultures (bacterial count [OD_{650}], HA assay [FHA-HA and PT-HA])
Crude purification[b)]	Filtrate (SDS-PAGE), [redacted] (SDS-PAGE), Supernatant after low-speed centrifugation (SDS-PAGE, HPLC analysis)
High purification[b)]	Supernatant after ultracentrifugation (SDS-PAGE, HPLC analysis), [redacted] (SDS-PAGE, HPLC analysis), Highly purified bulk (SDS-PAGE, HPLC analysis, purity test [FHA-HA and PT-HA])
Detoxification[b)]	Bulk before detoxification (SDS-PAGE, HPLC analysis, N-terminal amino acid sequence analysis[c)], internal amino acid sequence analysis[c)]), Validation of detoxification conditions (mouse histamine sensitization test, potency test)
Final filtration[b)]	Bulk of purified pertussis vaccine (SDS-PAGE, HPLC analysis, mouse histamine sensitization test, potency test, test for protein nitrogen content, pH, test for formaldehyde content)

a) 3 lots b) 6 lots c) 1 lot

(c) Adventitious agents safety evaluation

The raw materials of animal origin used in the production of the bulk of purified pertussis vaccine are as shown in Table 2-4.

Table 2-4. Raw materials of animal origin used in the production of bulk of purified pertussis vaccine

Process step	Raw material	Animal species	Specific part of animal used	Country of origin
MS	Skim milk	Bovine	Milk	Under inquiry[a)] (US, Australia, New Zealand)[b)]
	Polypepton	Bovine	Milk	China, Poland
	Pancreatin	Porcine	Pancreas	
	Defibrinated bovine blood	Bovine	Blood	Japan
	Casamino acids	Bovine	Milk	Under inquiry
WS and seed culture	Polypepton	Bovine	Milk	New Zealand
	Pancreatin	Porcine	Pancreas	
	Defibrinated bovine blood	Bovine	Blood	Australia, New Zealand
Seed culture and production culture	Casamino acids	Bovine	Milk	Australia, New Zealand

a) Used in the storage medium for the prepared MS.
b) To be used in the storage medium for future MS.

All raw materials are used as media components and all raw materials except for defibrinated bovine blood are autoclaved before use. Virus reduction factor of the detoxification step of the manufacturing process was as shown in Table 2-5.

Table 2-5. Virus reduction factor (log_{10}) of detoxification step (50 days)

Virus	Influenza virus (H3N2)	Herpes simplex virus type 1 (HSV-1)	Poliovirus type 1 (Sabin strain)
Virus reduction factor (log_{10})	> 5.28	> 4.29	> 6.125

(d) Manufacturing process development

In order to ensure consistent production of the pertussis bulk over a long period of time, a two-tiered seed lot system consisting of MS and WS was introduced as a means of seed control

during the development phase, and it was decided to use a newly prepared WS for the production of the pertussis bulk thereafter. Based on comparison of the results of specification tests and in-process tests, the pre- and post-change products were determined to be comparable. Furthermore, the pertussis bulk and the bulk before detoxification were analyzed by SDS-PAGE, Western blot, and HPLC. Based on comparison of analysis results, the pre- and post-change products were determined to be comparable.

2.A.(2).2) Characterization

The bulk of purified pertussis vaccine was characterized by SDS-PAGE, Western blot, HPLC, and spectroscopy.

The bands apparently representing FHA (220 kDa) and PT subunits (24 kDa and 13 kDa) were detected on SDS-PAGE. A 220 kDa protein was detected by anti-FHA monoclonal antibody and a 24 kDa protein was detected by anti-PT monoclonal antibody on Western blot. Thus, the bands were identified as FHA or PT. There were no differences in the HPLC chromatogram pattern between the bulk before detoxification and the bulk of purified pertussis vaccine. There were no lot-to-lot differences in the spectrum between the wavelengths of 240 nm and 340 nm.

2.A.(2).3) Impurities

For three lots of the bulk of purified pertussis vaccine, the residual *Bordetella pertussis* DNA level was demonstrated to be reduced to [redacted]% to [redacted]% in the highly purified bulk produced through Process flow I and [redacted]% to [redacted]% in the highly purified bulk produced through Process flow II and <[redacted]% in the pertussis bulk produced through Process flows I and II.

As process-related impurities, the removal of media derived components, Impurity A, Impurity B, Impurity C, Impurity D, and Impurity E was investigated using the bulk of purified pertussis vaccine. As a result, the level of media derived components was reduced to <[redacted]μg/mL in terms of bovine serum, the residual level of Impurity A was reduced to <[redacted]% of the amount added in the crude purification step, the level of Impurity B was reduced to ≤[redacted] μg/mL, the residual level of Impurity C was reduced to [redacted] to [redacted]μg/mL, the residual level of Impurity D was reduced to [redacted] to [redacted]μg/mL, and the residual level of Impurity E was reduced to [redacted] to [redacted]μg/mL.

Endotoxins were demonstrated to be reduced to <[redacted]EU/mL.

2.A.(2).4) Specifications

The specifications for the pertussis bulk include sterility test, inactivation test, test for the absence of residual activity of heat-labile toxin, bacterial endotoxins test, mouse histamine sensitization test, test for pH, test for formaldehyde content, test for freedom from abnormal toxicity, test for protein nitrogen content, and potency test.

2.A.(2).5) Standards

As the standards, the Standard Pertussis Vaccine and the Reference Pertussis Vaccine (for toxicity testing) distributed by NIID are used in the potency test and in the mouse histamine sensitization test, respectively. Each standard is stored at 2°C to 8°C. The Reference Standard Endotoxin is obtained from the Pharmaceutical and Medical Device Regulatory Science Society of Japan and stored at ≤8°C.

2.A.(2).6) Stability

Stability studies on the pertussis bulk are as shown in Table 2-6.

Table 2-6. Stability studies on pertussis bulk

Study	No. of lots[a)]	Temperature	Storage period
Long-term[b)]	3	[redacted] ± [redacted]°C	[redacted] months
Accelerated[b)]	3	25 ± 2°C/60 ± 5%RH	[redacted] months
Stress[c)]	1	37 ± 2°C/75 ± 5%RH	[redacted] days
Photostability[d)]		25 ± 2°C/60 ± 5%RH, 1500 ± 200 lx	[redacted] hours

a) Pertussis bulk produced at a commercial scale, using a one-tiered seed lot system
b) In addition to the specification tests for pertussis bulk, mouse body weight decreasing toxicity test, characterization test (description), staining test, and mouse leukocytosis promotion test were performed.
c) Sterility test, pH, test for formaldehyde content, test for protein nitrogen content, potency test, characterization test, SDS-PAGE, and HPLC
d) Sterility test, pH, test for formaldehyde content, potency test, test for protein nitrogen content, characterization test, SDS-PAGE, and HPLC

The samples met all the specifications during the storage period in the long-term stability study. Although failure to meet the acceptance criteria for potency at [redacted] months in the accelerated study and changes in the potency of the bulk material in the photostability study were observed, there was no failure to meet the acceptance criteria or significant change during storage for other attributes tested. Based on the above results, a shelf life of [redacted] years has been proposed for the pertussis bulk when it is stored at [redacted] ± [redacted]°C.

2.A.(3) Diphtheria bulk (Bulk diphtheria toxoid)

The diphtheria bulk is an antigen solution containing diphtheria toxoid produced by toxoiding of diphtheria toxin with formaldehyde solution.

2.A.(3).1) Manufacturing process

(a) Preparation and control of seeds

The Park-Williams No.8 strain of *Corynebacterium diphtheriae* distributed by NIID was passaged [redacted] times to establish a MS in 20[redacted] and the MS was passaged [redacted] times to prepare a WS. The MS and WS conformed to the tests listed in Table 2-7 and the seeds were qualified.

Table 2-7. Control tests on MS and WS of *Corynebacterium diphtheriae*

Test	MS	WS
Viable count assay	○	○
Identification test (identification by biochemical properties [enzyme activity, carbohydrate metabolism])	○	○
Staining test (Gram's method)	○	○
Test for presence of contaminating bacteria (contaminating bacterial growth in [redacted] medium and [redacted] medium supplemented with [redacted])	○	○
Culture assay (growth of the cultures as measured by OD_{590})	○	○
Antigen production assay (toxoid concentration [Lf/mL])[a)]	○	○
Antigen production rate (ELISA, the percentage of diphtheria toxoid-producing bacteria)	○	○
Nucleotide sequencing of the antigen gene	○	○

○: Tested, －: Not tested

a) Assay for diphtheria toxoid based on the precipitation reaction with the Reference Diphtheria Antitoxin in tubes (by varying the amount of antibody)

The MS has been stored at ≤[redacted]°C and the WS has been stored at ≤[redacted]°C. Stability during storage will be assessed by periodically performing the tests listed in Table 2-7 excluding culture assay. In addition, viable count assay will be performed at time of use of the MS or WS. When the number of remaining ampoules of the MS or WS is decreased to a certain level, a new MS will be prepared from the MS established in 20[redacted] and a new WS will be prepared from the new MS. The newly prepared MS or WS will be qualified by the tests listed in Table 2-7.

(b) Manufacturing process and critical steps/critical intermediates and process validation

The manufacturing process for the bulk diphtheria toxoid is as shown in Table 2-8.

Table 2-8. Summary of manufacturing process and controls for bulk diphtheria toxoid

	Manufacturing process	Intermediate	In-process testing
Cultivation	Seed culture [redacted] platinum loop of WS ↓ [redacted]mL, [redacted]±[redacted]°C, [redacted] hours, under static condition ↓ [redacted]mL, [redacted]±[redacted]°C, [redacted] hours, shaking		
	Production culture (main culture) [redacted]–[redacted]L, [redacted]±[redacted]°C, [redacted]–[redacted] hours, agitation	Cultures	Staining
	↓		
Crude purification	Filtration (pore size [redacted]–[redacted]μm)	Toxin solution	Lf test
	Concentration (Molecular weight cutoff [redacted]–[redacted])		
	Filtration (pore size [redacted]μm)	Crude purified toxin solution	
	↓		
Purification	[redacted]	[redacted]	
	Salt precipitation-1 ([redacted]%[redacted]) Recovery of precipitate	Salt precipitation-1 solution	
	Salt precipitation-2 ([redacted]%[redacted]) Recovery of supernatant	Salt precipitation-2 solution	
	Dialysis-1	Dialysis-1 solution	
	Column purification [redacted]chromatography	Column purified fraction	Purity[a)]
		Fraction pool	
	Dialysis-2	Dialysis-2 solution	
	Sterile filtration (pore size [redacted]μm)	Purified toxin solution	Lf test, Purity[a)]
	↓		
Toxoiding	Toxoiding: [redacted]vol % formalin	Toxoid solution	
	Dialysis (Molecular weight cutoff [redacted])	Dialyzed toxoid solution	Lf test
	Sterile filtration (pore size [redacted]μm)	Diphtheria bulk (bulk diphtheria toxoid)	Detoxification of diphtheria toxin (guinea pigs, rabbits), Filter integrity

[redacted]: Critical steps or critical intermediates

a) Quantity of diphtheria toxin per mg of protein nitrogen (Lf)

The diphtheria bulk or the intermediates in the manufacturing process for the diphtheria bulk were evaluated for the parameters listed in Table 2-9. The test results demonstrated that each process step is adequately controlled to ensure consistent production.

Table 2-9. Process validation/evaluation of manufacturing process for bulk diphtheria toxoid

Process step	Parameter
Cultivation[a)]	Cultures (turbidity [OD_{590}], toxin content [Lf test])
Crude purification[b)]	Toxin solution (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)]), Crude purified toxin solution (Lf test[a)], test for protein nitrogen content[a)])
Purification[b)]	[redacted] (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)]), Salt precipitation-1 solution and Salt precipitation-2 solution (Lf test[a)], test for protein nitrogen content[a)]), Dialysis-1 solution (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)]), Column purified fraction (purity test[a)], SDS-PAGE[b)], HPLC analysis[b)]), Purified toxin solution (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)], N-terminal amino acid sequence analysis[c)])
Toxoiding	Validation of toxoiding conditions[c)] (test for detoxification of diphtheria toxin [rabbits])
Diphtheria bulk[d)]	Bulk diphtheria toxoid (purity test, potency test [fluid, adsorbed], osmolality, pH, test for formaldehyde content)

a) 3 lots b) 6 lots c) 1 lot d) 9 lots

(c) Adventitious agents safety evaluation

The raw materials of animal origin used in the production of the bulk diphtheria toxoid are as

shown in Table 2-10.

Table 2-10. Raw materials of animal origin used in the production of bulk diphtheria toxoid

Process step	Raw material	Animal species	Specific part of animal used	Country of origin
MS	Skim milk	Bovine	Milk	US, Australia, New Zealand[a)]
	Equine serum	Equine	Blood	
	Peptone	Bovine	Milk	New Zealand
	Pancreatin	Porcine	Pancreas/Duodenum	
WS and seed culture	Equine serum	Equine	Blood	
	Peptone	Bovine	Milk	New Zealand
	Pancreatin	Porcine	Pancreas/Duodenum	
Seed culture and production culture	Beef digest	Bovine	Muscle	Australia

a) Used in the storage medium for prepared MS.

All raw materials are used as media components and all raw materials excluding equine serum and beef digest are autoclaved before use. Virus reduction factor of the toxoiding step of the manufacturing process was as shown in Table 2-11.

Table 2-11. Virus reduction factor of toxoiding step (31 days) (log_{10})

Virus	Influenza virus (H3N2)	Herpes simplex virus type 1 (HSV-1)	Poliovirus type 1 (Sabin strain)
Virus reduction factor (log_{10})	> 5.8	> 4.19	> 6.25

(d) Manufacturing process development

In order to ensure consistent production of the diphtheria bulk over a long period of time, a two-tiered seed lot system consisting of MS and WS was introduced as a means of seed control during the development phase and it was decided to use a newly prepared WS for the production of the diphtheria bulk thereafter. Based on comparison of the results of specification tests and in-process tests, the pre- and post-change products were determined to be comparable. Furthermore, the diphtheria bulk and the purified toxin solution were analyzed by SDS-PAGE, Western blot, and HPLC. Based on comparison of analysis results, the pre- and post-change products were determined to be comparable.

2.A.(3).2) Characterization

The bulk diphtheria toxoid was characterized by SDS-PAGE, Western blot, HPLC analysis, and spectroscopy.

A band apparently representing a protein derived from diphtheria toxin (58 kDa) was detected on SDS-PAGE and a 58 kDa protein was detected by anti-diphtheria toxin monoclonal antibody on Western blot and the band was identified as diphtheria toxin. [redacted] main peaks were detected by HPLC. There were no lot-to-lot differences in the spectrum between the wavelengths of 240 nm and 340 nm.

2.A.(3).3) Impurities

For three lots of the bulk diphtheria toxoid, the residual *Corynebacterium diphtheriae* DNA level was demonstrated to be reduced to [redacted]% to [redacted]% after the purification process and <[redacted]% in the diphtheria bulk.

As process-related impurities, the removal of media derived components, Impurity A, and Impurity E was investigated using the bulk diphtheria toxoid. As a result, the level of media derived components was reduced to <[redacted]μg/mL in terms of bovine serum, the residual level of Impurity A was reduced to <[redacted]% of the amount added in the purification process, and the residual level of Impurity E was reduced to [redacted] to [redacted]μg/mL.

Endotoxins were demonstrated to be reduced to <[redacted]EU/mL.

2.A.(3).4) Specifications

The specifications for the bulk diphtheria toxoid include sterility test, purity test, test for detoxification of diphtheria toxin (guinea pigs), test for detoxification of diphtheria toxin (rabbits), test for formaldehyde content, test for freedom from abnormal toxicity, potency test, and bacterial endotoxins test.

2.A.(3).5) Standards and reference materials

As the standards, the Reference Diphtheria Antitoxin (for flocculation test) distributed by NIID is used in the purity test and the Standard Diphtheria Toxoid and the Standard Diphtheria Antitoxin distributed by NIID are used in the potency test and these standards are stored at 2°C to 8°C.

2.A.(3).6) Stability of diphtheria bulk

Stability studies on the diphtheria bulk are as shown in Table 2-12.

Table 2-12. Stability studies on diphtheria bulk

Study	No. of lots[a)]	Temperature	Storage period
Long-term[b)]	3	[redacted] ± [redacted]°C	[redacted] months
Accelerated[b)]	3	25 ± 2°C/60 ± 5%RH	[redacted] months
Stress[c)]	1	37 ± 2°C/75 ± 5%RH	[redacted] days
Photostability[c)]		25 ± 2°C/60 ± 5%RH, 1500 ± 200 lx	[redacted] hours

a) Diphtheria bulk produced at a commercial scale, using a one-tiered seed lot system
b) The specification tests for the diphtheria bulk (excluding bacterial endotoxins test), test for pH, and characterization test (visual description) were performed.
c) Purity test, sterility test, test for pH, test for formaldehyde content, potency test, characterization test, SDS-PAGE, and HPLC were performed.

In the long-term stability study, though formaldehyde content tended to decrease, the samples

met all the specifications during the storage period. Although 1 lot failed to meet the acceptance criteria for potency at [redacted] months (the same lot met the acceptance criteria at [redacted] and [redacted] months) in the accelerated study and formaldehyde content tended to decrease in the stress study, there was no failure to meet the acceptance criteria or significant change during storage for other attributes tested. Based on the above results, a shelf life of [redacted] years has been proposed for the diphtheria bulk when it is stored at [redacted]±[redacted]°C.

2.A.(4) Tetanus bulk (Bulk tetanus toxoid)

The tetanus bulk is an antigen solution containing tetanus toxoid produced by toxoiding of tetanus toxin with formaldehyde solution.

2.A.(4).1) Manufacturing process

(a) Preparation and control of seeds

Harvard strain of *Clostridium tetani* distributed by NIID was passaged. Then, a colony was selected based on the amount of antigen produced and further passaged [redacted] times to establish a MS in 20[redacted]. The MS was passaged [redacted] times to prepare a WS. The MS and WS conformed to the tests listed in Table 2-13 and the seeds were qualified.

Table 2-13. Control tests on MS and WS of *Clostridium tetani*

Test	MS	WS
Viable count assay	○	○
Identification test (identification by biochemical properties [enzyme activity, carbohydrate metabolism])	○	○
Staining test (Gram's method)	○	○
Test for presence of contaminating bacteria (contaminating bacterial growth in [redacted] medium and [redacted] medium)	○	○
Culture assay (growth of the cultures as measured by OD_{590})	○	○
Antigen production assay (tetanus antigen concentration [Lf/mL])[a)]	○	○
Antigen production rate (ELISA, the percentage of tetanus antigen-producing bacteria)	○	○
Nucleotide sequencing of the antigen gene	○	○
L_+ test (assay for tetanus toxin activity in mice)	○	○

○: Tested, －: Not tested

a) Precipitation reaction with the Reference Tetanus Antitoxin in tubes (by varying the amount of antibody)

The MS and WS have been stored at ≤[redacted]°C and stability during storage will be assessed by periodically performing the tests listed in Table 2-13, excluding culture assay. In addition, viable count assay will be performed at time of use of the MS or WS. When the number of remaining ampoules of the MS or WS is decreased to a certain level, a new MS will be prepared from the MS established in 20[redacted] and a new WS will be prepared from the new MS. The newly prepared MS or WS will be qualified by the tests listed in Table 2-13.

(b) Manufacturing process and critical steps/critical intermediates and process validation

The manufacturing process for the bulk tetanus toxoid is as shown in Table 2-14.

Table 2-14. Summary of manufacturing process and controls for bulk tetanus toxoid

Manufacturing process		Intermediate	In-process testing
Cultivation	Seed culture WS [redacted] mL ↓ [redacted] mL, [redacted] ± [redacted] °C, [redacted] hours, under static condition ↓ [redacted] mL, [redacted] ± [redacted] °C, [redacted] hours, under static condition		
	Production culture (main culture) [redacted] L, [redacted] ± [redacted] °C, [redacted] days, agitation	Cultures	Staining
↓			
Crude purification	Sterile filtration (pore size [redacted] - [redacted] μm)	Toxin solution	Lf test
	Concentration (Molecular weight cutoff [redacted])		
	Sterile filtration (pore size [redacted] μm)	Crude purified toxin solution	
↓			
Purification	Salt precipitation-1 ([redacted] % [redacted]) Recovery of precipitate	Salt precipitation-1 solution	
	[redacted]	[redacted]	
	Salt precipitation-2 ([redacted] % [redacted]) Recovery of supernatant	Salt precipitation-2 solution	
	Dialysis-1 (Molecular weight cutoff [redacted])	Dialysis-1 solution	
	Sterile filtration (pore size [redacted] μm)	MF filtrate	
	Column purification [redacted] chromatography	Column purified fraction	Purity[a)]
		Fraction pool	
	Dialysis-2 (Molecular weight cutoff [redacted])	Dialysis-2 solution	
	Sterile filtration (pore size [redacted] μm)	Purified toxin solution	Lf test, Purity[a)]
↓			
Toxoiding	Toxoiding: [redacted] % formalin [redacted] - [redacted] °C: [redacted] days, [redacted] ± [redacted] °C: [redacted] days	Solution before toxoiding Toxoid solution	
	Dialysis (Molecular weight cutoff [redacted])	Dialyzed toxoid solution	Lf test
	Sterile filtration (pore size [redacted] μm)	Tetanus bulk (Bulk tetanus toxoid)	Detoxification of tetanus toxin Filter integrity

[redacted]: Critical steps or critical intermediates

a) Quantity of tetanus toxin (Lf) per mg of protein nitrogen

The tetanus bulk or the intermediates in the manufacturing process for the tetanus bulk were evaluated for the parameters listed in Table 2-15. The test results demonstrated that each process step is adequately controlled to ensure consistent production.

Table 2-15. Process validation/evaluation of manufacturing process for bulk tetanus toxoid

Process step	Parameter
Cultivation[a)]	Cultures (toxin content [Lf test])
Crude purification[b)]	Crude purified toxin solution (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)])
Purification[b)]	MF filtrate (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)]), Column purified fraction (Lf test[a)], test for protein nitrogen content[a)]), Purified toxin solution (Lf test[a)], test for protein nitrogen content[a)], SDS-PAGE[b)], HPLC analysis[b)], N-terminal amino acid sequence analysis[c)])
Toxoiding	Validation of toxoiding conditions[c)] (detoxification test)
Tetanus bulk[d)]	Bulk tetanus toxoid (purity test, potency test [fluid, adsorbed], osmolality, pH, test for formaldehyde content)

a) 3 lots b) 6 lots c) 1 lot d) 10 lots

(c) Adventitious agents safety evaluation

The raw materials of animal origin used in the production of the bulk tetanus toxoid are as shown in Table 2-16.

Table 2-16. Raw materials of animal origin used in the production of bulk tetanus toxoid

Process step	Raw material	Animal species	Specific part of animal used	Country of origin
MS	Beef	Bovine	Muscle	Australia, New Zealand
	Polypepton	Bovine	Milk	New Zealand
	Pancreatin	Porcine	Pancreas, Duodenum	
	Bovine liver	Bovine	Liver	Australia, New Zealand
WS and seed culture	Beef	Bovine	Muscle	Australia, New Zealand
	Polypepton	Bovine	Milk	New Zealand
	Bovine liver	Bovine	Liver	Australia, New Zealand
	Pancreatin	Porcine	Pancreas, Duodenum	
Seed culture and production culture	Polypepton	Bovine	Milk	New Zealand
	Beef heart infusion	Bovine	Heart	Australia, New Zealand
	Pancreatin	Porcine	Pancreas, Duodenum	

All raw materials are used as media components and autoclaved before use. Virus reduction factor of the toxoiding step of the manufacturing process is as shown in Table 2-17.

Table 2-17. Virus reduction factor of toxoiding step (17 days) (log_{10})

Virus	Influenza virus (H3N2)	Herpes simplex virus type 1 (HSV-1)	Poliovirus type 1 (Sabin strain)
Virus reduction factor (log_{10})	> 5.61	> 4.29	> 6.167

(d) Manufacturing process development

In order to ensure consistent production of the tetanus bulk over a long period of time, a two-tiered seed lot system consisting of MS and WS was introduced as a means of seed control during the development phase, and it was decided to use a newly prepared WS for the production of the tetanus bulk thereafter. Based on comparison of the results of specification tests and in-process tests, the pre- and post-change products were determined to be comparable. Furthermore, the tetanus bulk and the purified toxin solution were analyzed by SDS-PAGE, Western blot, and HPLC and based on comparison of analysis results, the pre- and post-change products were determined to be comparable.

2.A.(4).2) Characterization

The bulk tetanus toxoid was characterized by SDS-PAGE, Western blot, HPLC analysis, and spectroscopy.

A band apparently representing a protein derived from tetanus toxin (150 kDa) was detected on SDS-PAGE. A 150 kDa protein was detected by anti-tetanus toxin monoclonal antibody on

Western blot. Thus, the band was identified as tetanus toxin. [redacted] peaks derived from tetanus toxin were detected by HPLC. There were no lot-to-lot differences in the spectrum between the wavelengths of 240 nm and 340 nm.

2.A.(4).3) Impurities

For three lots of the bulk tetanus toxoid, the residual *Clostridium tetani* DNA level was demonstrated to be reduced to [redacted]% to [redacted]% after the purification process and <[redacted]% in the tetanus bulk.

As process-related impurities, the removal of media derived components, Impurity A, and Impurity E was investigated using the bulk tetanus toxoid. As a result, the level of media derived components was reduced to <[redacted]μg/mL in terms of bovine serum, the residual level of Impurity A was reduced to <[redacted]% of the amount added in the purification process, and the residual level of Impurity E was reduced to [redacted] to [redacted]μg/mL.

Endotoxins were demonstrated to be reduced to ≤[redacted]EU/mL.

2.A.(4).4) Specifications

The specifications for the bulk tetanus toxoid include purity test, sterility test, test for detoxification of tetanus toxin, test for formaldehyde content, test for freedom from abnormal toxicity, potency test, and bacterial endotoxins test.

2.A.(4).5) Standards and reference materials

As a reference preparation, the Reference Tetanus Antitoxin (for flocculation test) distributed by NIID is used in the Lf test and as the standard, the Standard Tetanus Toxoid distributed by NIID is used in the potency test. Both are stored at 2°C to 8°C.

2.A.(4).6) Stability of the tetanus bulk

Stability studies on the tetanus bulk are as shown in Table 2-18.

Table 2-18. Stability studies on tetanus bulk

Study	No. of lots[a)]	Temperature	Storage period
Long-term[b)]	3	[redacted] ± [redacted] °C	[redacted] months
Accelerated[b)]	3	25 ± 2°C/60 ± 5%RH	[redacted] months
Stress[c)]	1	37 ± 2°C/75 ± 5%RH	[redacted] days
Photostability[c)]		25 ± 2°C/60 ± 5%RH, 1500 ± 200 lx	[redacted] hours

a) Tetanus bulk produced at a commercial scale, using a one-tiered seed lot system

b) In addition to the specification tests for the tetanus bulk (excluding bacterial endotoxins test), test for pH and characterization test (visual description) were performed.

c) Purity test, sterility test, test for pH, test for formaldehyde content, potency test, characterization test, SDS-PAGE, and HPLC analysis were performed.

The samples met all the specifications during the storage period in the long-term stability study. Although variations in potency were noted, there was no failure to meet the acceptance criteria or significant change during storage for other attributes tested. Based on the above, a shelf life of [redacted] years has been proposed for the tetanus bulk when it is stored at [redacted] ± [redacted] °C.

2.A.(5) Drug product

2.A.(5).1) Description and composition of the drug product and formulation development

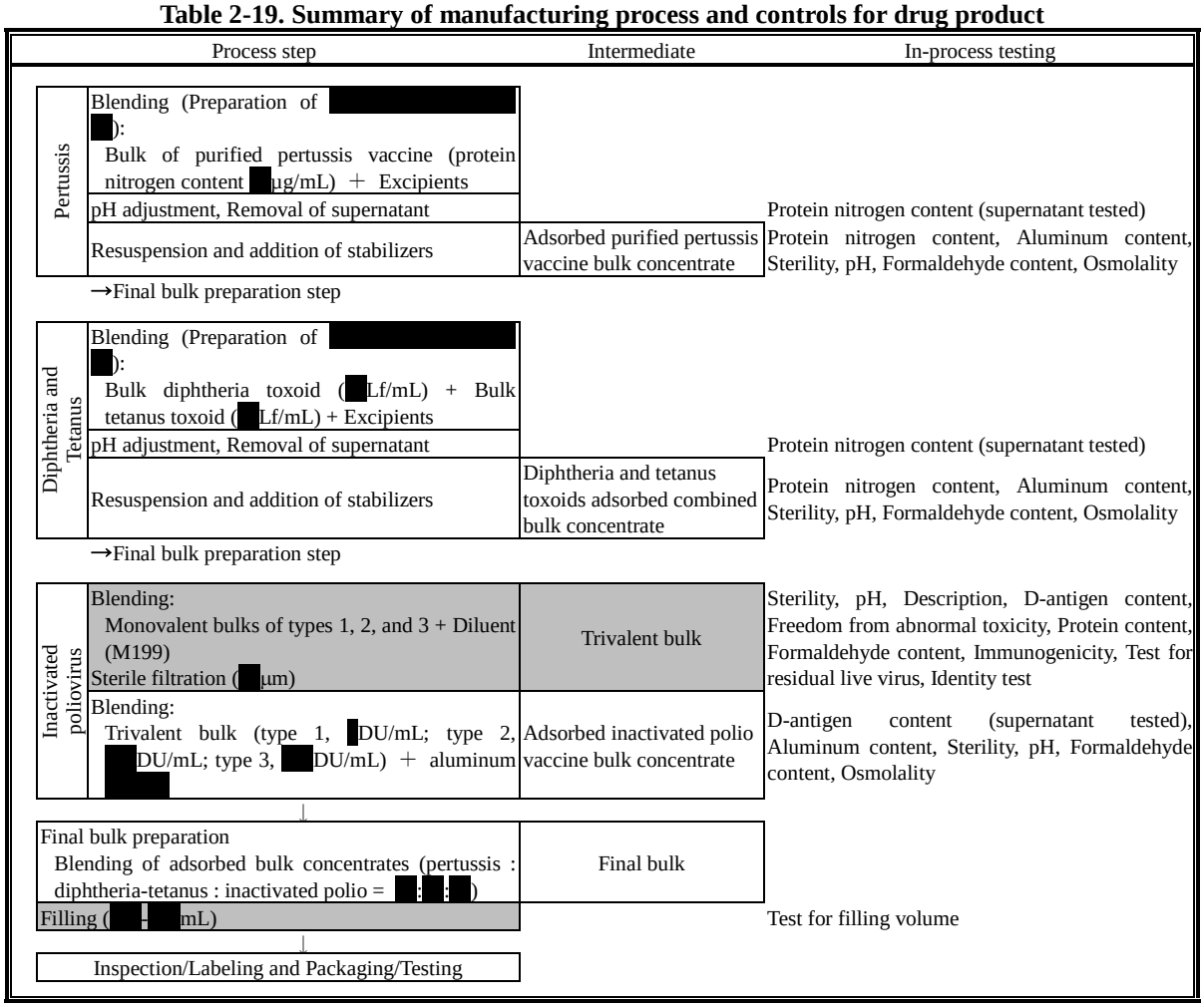
Each 0.5-mL dose of the drug product contains ≥4 units of the *Bordetella pertussis* protective antigen, ≤15 Lf of diphtheria toxoid, and ≤2.5 Lf of tetanus toxoid and 1.5 D-antigen units (DU) of inactivated poliovirus type 1 (Sabin strain), 50 DU of inactivated poliovirus type 2 (Sabin strain), and 50 DU of inactivated poliovirus type 3 (Sabin strain) as active ingredients and 0.08 mg of aluminum [redacted] and 0.02 mg of aluminum hydroxide as adjuvants. Each dose also contains 0.025 mg of formaldehyde, 0.0175 mg of disodium edetate hydrate, M199, buffering agents, an isotonizing agent, and pH adjusting agents as excipients. The drug product is available in glass prefilled syringes.

2.A.(5).2) Manufacturing process

(a) Manufacturing process

The manufacturing process for the drug product is as shown in Table 2-19.

Table 2-19. Summary of manufacturing process and controls for drug product



: Critical steps or critical intermediates

(b) Manufacturing process development

Three formulations with different inactivated poliovirus D-antigen contents (Formulation H, Formulation M, Formulation L) were used during product development, and based on clinical study data, Formulation M has been proposed for registration (Table 2-20).

Table 2-20. Quantities of active ingredients in 0.5 mL of DPT-sIPV under development or approved DPT

Ingredient	DPT	Formulation H	Formulation M	Formulation L
Bordetella pertussis protective antigen	≥ 4 units	≥ 4 units		
Diphtheria toxoid	≤ 15 Lf	≤ 15 Lf		
Tetanus toxoid	≤ 2.5 Lf	≤ 2.5 Lf		
Inactivated poliovirus type 1	－	3 DU	1.5 DU	0.75 DU
Inactivated poliovirus type 2	－	100 DU	50 DU	25 DU
Inactivated poliovirus type 3	－	100 DU	50 DU	25 DU

－: Not contained

2.A.(5).3) Specifications

The specifications for the drug product include test for pH, test for aluminum content, test for

formaldehyde content, sterility test, test for freedom from abnormal toxicity, bacterial endotoxins test, mouse body weight decreasing toxicity test, mouse histamine sensitization test, test for detoxification of diphtheria toxin, test for detoxification of tetanus toxin, potency test (pertussis), potency test (diphtheria), potency test (tetanus), rat immunogenicity test, description test, test for osmolality, content uniformity test, test for extractable volume, foreign insoluble matter test, insoluble particulate matter test, test for D-antigen content, and identity test.

2.A.(5).4) Reference materials

In addition to the standards used in the tests for the drug substance, the Reference Adsorbed Diphtheria Toxoid (for Combined Vaccine) (for the potency test [diphtheria]) and the Reference Adsorbed Tetanus Toxoid (for Combined Vaccine) (for the potency test [tetanus]) distributed by NIID are used in the specification tests for the drug product and stored at 2°C to 8°C or ≤ −80°C. As reference materials, the Reference Preparation for IPV Potency Testing supplied by NIID is used in the rat immunogenicity test and the standard viruses distributed by the Japan Poliomyelitis Research Institute are used in the test for D-antigen content and the Reference Preparation for IPV Potency Testing is stored at ≤ −70°C and the standard viruses are stored at ≤ −60°C.

2.A.(5).5) Stability

Stability studies on the drug product are as shown in Table 2-21.

Table 2-21. Stability studies on drug product

Study	No. of lots[a)]	Temperature	Storage period
Long-term[b)]	3	10 ± 2°C	[redacted] months
Accelerated[b)]	3	25 ± 2°C/60 ± 5%RH	6 months
Stress (Formulation H)[c)]	1	37 ± 2°C/75 ± 5%RH	21 days
Photostability (Formulation H)[c)]		25 ± 2°C/60 ± 5%RH, 7000 ± 200 lx	21 hours
Photostability (Formulation H)[d)]		25 ± 2°C/60 ± 5%RH, 1500 ± 200 lx	48 hours

a) Drug product produced at a pilot scale, using a one-tiered seed lot system
b) The specification tests for the drug product (excluding identity test) and test for protein nitrogen content and testing of container for airtightness were performed.
c) Test for pH, test for formaldehyde content, sterility test, potency tests (pertussis, diphtheria, tetanus), test for D-antigen content, rat immunogenicity test, description test, and test for protein content were performed.
d) Test for D-antigen content and rat immunogenicity test were performed.

At the time of regulatory submission, long-term data up to 12 months (up to 18 months for 1 of 3 lots) were submitted. The data demonstrated that the samples were within the specifications. Although D-antigen content tended to decrease at the accelerated condition and Formulation H tended to show lower immunogenicity in rats in the photostability studies, there was no failure to meet the acceptance criteria or significant change during storage for other attributes tested. Based on the above results, a shelf life of 12 months has been proposed for the proposed commercial drug product (Formulation M) when it is stored at ≤10°C (avoid freezing),

protected from light.

2.B Outline of the review by PMDA

Although PMDA is asking the applicant to explain the details of the manufacturing process, controls for the product and raw materials of biological origin etc., based on the submitted data, PMDA considers that there are no significant quality problems that would affect the evaluation of non-clinical and clinical studies. The conclusion of the review by PMDA including the applicant's explanation is outlined in the Review Report (2).

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

As primary pharmacodynamic studies, potency tests and immunogenicity studies were conducted using Formulation H, Formulation M, and Formulation L [see "2.A.(5).2).(b) Manufacturing process development"]. Safety pharmacology studies with Formulation H to assess its effects on the central nervous system and respiratory system were conducted.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) Potency tests for *Bordetella pertussis* protective antigen, diphtheria toxoid, and tetanus toxoid (4.2.1.1.4, Study P[redacted]-12 and 21 other studies, Study PT34A-[redacted]05)

Potency was determined in accordance with the requirements of "Potency test for Adsorbed Purified Pertussis Vaccine," "Potency test for Adsorbed Diphtheria Toxoid," and "Potency test for Adsorbed Tetanus Toxoid" for the standard "Adsorbed Diphthera-Purified Pertussis-Tetanus Combined Vaccine" listed in the Minimum Requirements for Biological Products, which are used for determining the potency of the active ingredients (the DPT components) of Adsorbed Diphthera-Purified Pertussis-Tetanus Combined Vaccine (DPT) in Japan. As a result, the potency of the DPT components of the DPT-sIPV vaccine was similar to the potency of DPT and it was discussed that inactivated poliovirus does not affect the potency of the DPT components.

3.(i).A.(1).2) Inactivated poliovirus immunogenicity study in rats (4.2.1.1.5, Study PN[redacted]-01)

Rats (females) received a single intramuscular injection of 0.5 mL of Formulation H, Formulation M, or Formulation L (the samples were serially diluted to four dilutions; 10 rats/group for each dilution; a total of 120 rats in 12 groups) in the hindlimb thigh muscle and neutralizing antibody titers against attenuated strains of poliovirus types 1, 2, and 3 were

measured 21 days later. The relative pontency of the DPT-sIPV vaccine to the Reference Preparation for IPV Potency Testing [1] distributed by NIID [see "2.A.(5).4) Reference materials"] was calculated as the ratio of neutralizing antibody titers. The results were as shown in Table 3-1 and the relative potencies were greater than 1. Therefore, it was discussed that all formulations have equivalent or greater potency than the Reference Preparation for IPV Potency Testing.

Table 3-1. Relative potency to Reference Preparation for IPV Potency Testing

	Formulation H	Formulation M	Formulation L
Attenuated strain of poliovirus type 1	[redacted]	[redacted]	[redacted]
Attenuated strain of poliovirus type 2	[redacted]	[redacted]	[redacted]
Attenuated strain of poliovirus type 3	[redacted]	[redacted]	[redacted]

3.(i).A.(1).3) Inactivated poliovirus immunogenicity studies in cynomolgus monkeys (4.2.1.1.6, Study PT34A-[redacted]02, Study PT34A-[redacted]01)

Cynomolgus monkeys (5-6 females/group) were injected subcutaneously in the upper arm with 0.5 mL of Formulation H, the Reference Preparation for IPV Potency Testing, an inactivated polio vaccine derived from virulent strains of poliovirus (vIPV; study vaccine, foreign-marketed vaccine product A), or a DPT-vIPV combined vaccine (DPT-vIPV; study vaccine, foreign-marketed vaccine product B) (a total of 21 cynomolgus monkeys in four groups) at Weeks 0, 3, 6, and 33. Neutralizing antibody titers against attenuated and virulent strains of poliovirus types 1, 2, and 3 were measured at Weeks 0, 3, 6, 9, 33, and 36. Formulation H induced neutralizing antibody titers $\geq 2^9$ fold of all serotypes at Week 9 (at 3 weeks after the third dose) and Week 36 (at 3 weeks after the fourth dose). Although the neutralizing antibody titers against the virulent strain of poliovirus type 1 at Weeks 6 and 36 in the Formulation H group were lower than those found in the vIPV and DPT-vIPV groups, the measurements at other timepoints were similar. The geometric mean neutralizing antibody titers at the last timepoint in the study were as shown in Table 3-2 and the neutralizing antibody titers were $\geq 2^{10}$ fold in all of the Formulation H, vIPV, and DPT-vIPV groups. Based on the above, it was discussed that Formulation H, vIPV, and DPT-vIPV showed comparable immunogenicity.

Table 3-2. Geometric mean neutralizing antibody titers (log_2) at Week 36

	Attenuated strains of poliovirus			Virulent strains of poliovirus		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Formulation H	12.1	14.7	13.1	10.8	12.7	12.7
Reference Preparation for IPV Potency Testing	9.8	10.6	10.8	8.6	9.3	10.3
DPT-vIPV	12.8	13.7	13.2	13.2[a)]	13.5	12.7
vIPV	12.4	12.5	13.6	12.5[a)]	12.6	13.1

a) $P < 0.05$ (t-test was performed at the 5% level of significance to compare with DPT-sIPV [Formulation H])

1 Prepared to be of immunogenicity equivalent to the WHO reference preparation or a foreign-marketed inactivated polio vaccine derived from virulent strains of poliovirus (vIPV).

In the study, one cynomolgus monkey in the vIPV group died 20 days after the first dose and one cynomolgus monkey in the Formulation H group died 10 weeks after the third dose, but necropsy revealed no abnormal findings for both cases and the deaths were considered unrelated to study vaccine.

3.(i).A.(2) Safety pharmacology

As there were no abnormalities in ECG or pulse rate following the administration of Formulation H in a repeat-dose toxicity study in dogs, the DPT-sIPV vaccine is unlikely to affect the cardiovascular system and a safety pharmacology study to assess its effects on the cardiovascular system was not performed.

3.(i).A.(2).1) Effects on central nervous system (4.2.1.3.1, Study FBM[redacted]-2322)

Rats (8 males/group) received a single subcutaneous injection of 0.8 mL/kg of Formulation H or saline (a total of 16 rats in two groups; ≥10-fold the proposed clinical doses). General activity and behaviour were assessed by a functional observation battery (FOB) at pre-dose and 0.25, 2, and 6 hours post-dose. As a result, no effects on the central nervous system were observed.

3.(i).A.(2).2) Effects on respiratory system (4.2.1.3.3, Study FBM[redacted]-2323)

Rats (8 males/group) received a single subcutaneous injection of 0.8 mL/kg of Formulation H or saline (a total of 16 rats in two groups; ≥10-fold the proposed clinical doses). Tidal volume, respiratory rate, and minute ventilation were measured at pre-dose and 0.25, 1, 2, 4, and 6 hours post-dose, and no effects on the respiratory system were observed.

3.(i).B Outline of the review by PMDA

Primary pharmacodynamic studies conducted by the applicant demonstrated that the DPT-sIPV vaccine induces neutralizing antibodies against poliovirus. Also, according to the submitted literature, inactivated poliovirus types 1, 2, and 3 prepared from Sabin strains, as with the candidate DPT-sIPV vaccine, provided protection against polio in poliovirus-susceptible transgenic mice, suggesting the association between neutralizing antibody against poliovirus and protection from polio (*J Infect Dis*. 1997;175:441-444, *J Infect Dis*. 2004;190:1404-1412, *J Infect Dis*. 2006;194:804-807). Therefore, PMDA concluded that the candidate vaccine can be expected to be protective against polio. However, PMDA considers that when it was decided to conduct a confirmatory study with Formulation M, an immunogenicity study using Formulation M in cynomolgus monkeys should have been conducted to compare and discuss on the neutralizing antibodies induced by Formulation M vs. vIPV and DPT-vIPV, which have already been marketed overseas.

3.(ii) Summary of pharmacokinetic studies

No pharmacokinetic studies have been conducted.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

As toxicity studies of the DPT-sIPV vaccine, single-dose toxicity, repeat-dose toxicity, genotoxicity, and local tolerance studies were conducted using two formulations with different inactivated poliovirus D-antigen contents [Formulation H and Formulation M, see "2.A.(5).2).(b) Manufacturing process development"].

3.(iii).A.(1) Single-dose toxicity (4.2.3.1.1, Study FBM [redacted]-2317; 4.2.3.1.2, Study FBM [redacted]-4318)

Rats (5 rats/sex/group) and beagle dogs (2 males/group) were subcutaneously injected with saline or 0.8 mL/kg or 4 mL/kg of Formulation H (a total of 30 rats in three groups and a total of 6 beagle dogs in three groups). As a result, no deaths occurred in any group and the approximate lethal dose was considered to be >4 mL/kg for both rats and beagle dogs. When 0.8 mL/kg of Formulation H was administered, the doses of the *Bordetella pertussis* protective antigen, diphtheria toxoid, and tetanus toxoid were approximately 10 times the proposed clinical doses and the doses of inactivated poliovirus types 1, 2, and 3 were approximately 20 times the proposed clinical doses.

3.(iii).A.(2) Repeat-dose toxicity (4.2.3.2.1, Study FBM [redacted]-2319; 4.2.3.2.2, Study P[redacted]0336; 4.2.3.2.3, Study FBM [redacted]-4320; 4.2.3.2.4, Study B[redacted]1122)

Rats (10 rats/sex/group) received four doses of saline or 0.8 mL/kg of Formulation H by subcutaneous injection at weekly intervals (a total of 40 rats in two groups). As a result, although there were no deaths or systemic toxicological signs, pathological findings included pale yellowish-white nodules representing foreign-body granulomas in the subcutaneous tissue at the injection site in all rats in the Formulation H group. Rats (10 rats/sex/group) received five doses of 0.8 mL/kg of Formulation M or 0.8 mL/kg of Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine (DPT) by subcutaneous injection at the same site at weekly intervals (a total of 40 rats in two groups) for the evaluation of local tolerance. As a result, all rats in both groups exhibited moderate foreign-body granulomas at the injection site, which had resolved gradually during recovery periods.Cumulative irritation and its reversibility were considered similar for Formulation M and DPT.

Beagle dogs (3 beagle dogs/sex/group) received four doses of saline or 0.8 mL/kg of

Formulation H by subcutaneous injection at weekly intervals (a total of 12 beagle dogs in two groups). As a result, all beagle dogs in the Formulation H group exhibited foreign-body granulomas with the deposition of eosinophilic material in the subcutaneous tissue at the injection site, which tended to resolve. Blood biochemistry tests revealed that the percentage of albumin in serum protein fractions was significantly lower in males and tended to be lower in females and the percentage of γ-globulin tended to be higher in males and females in the Formulation H group. All beagle dogs in the Formulation H group had mild germinal center development in the spleen. Furthermore, a single case of polyarteritis in the chest occurred in 1 female in the Formulation H group. To assess the reproducibility of this finding, beagle dogs (3 females/group) received four doses of saline or 0.08, 0.8, or 4.0 mL/kg of Formulation H by subcutaneous injection at weekly intervals (a total of 12 beagle dogs in four groups). As a result, polyarteritis in the chest was not detected in any group. Also, it has been reported that naturally occurring polyarteritis is found sporadically in beagle dogs (*Toxicity Study Course 5. Toxicological pathology*. 1991:63-64, *Veterinary Pathology*. 1987;24:537-544, *Journal of Comparative Pathology*. 1987;97:121-128). Taking these findings into account, it was discussed that polyarteritis observed in 1 female in the Formulation H group was an incidental case unrelated to the DPT-sIPV vaccine.

As described above, except for effects at the injection site, there were no toxic changes that would affect the systemic condition following the administration of the DPT-sIPV vaccine.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1.1, Study FBM [redacted]-8324; 4.2.3.3.1.2, Study FBM [redacted]-8585; 4.2.3.3.1.3, Study FBM[redacted]-8325; 4.2.3.3.1.4, Study FBM [redacted]-8586)

Although bacterial reverse mutation assay and mouse lymphoma tk assay indicated that Formulation H was genotoxic, as there was no evidence of genotoxicity for Formulation H produced without the addition of formalin, it was discussed that the active ingredients of the DPT-sIPV vaccine have no genotoxic potential.

3.(iii).A.(4) Carcinogenicity

No carcinogenicity studies have been conducted. There were no findings of carcinogenic potential in repeat-dose toxicity studies.

3.(iii).A.(5) Reproductive and developmental toxicity

No reproductive and developmental toxicity studies have been conducted. There were no abnormalities in the reproductive organs in repeat-dose toxicity studies.

3.(iii).A.(6) Local tolerance (4.2.3.6, Study FBM [redacted]-3321)

Rabbits (6 males/group) received single intramuscular injections of 0.5 mL of Formulation H and 0.5 mL of saline into the right and left vastus lateralis muscle, single intramuscular injections of 1 mL of 0.425 w/v% acetic acid solution and 1 mL of 1.7 w/v% acetic acid solution into the right and left vastus lateralis muscle, or a single intramuscular injection of 0.5 mL of DPT into the right vastus lateralis muscle only (a total of 18 rabbits in three groups). As a result, like DPT, Formulation H was considered to be more locally irritating than saline and less locally irritating than 0.425 w/v% acetic acid solution and it was demonstrated that the local irritation of the DPT-sIPV vaccine is not increased by the addition of inactivated poliovirus.

3.(iii).B Outline of the review by PMDA

3.(iii).B.(1) Systemic toxicity assessment

The repeat-dose systemic toxicity of the DPT-sIPV vaccine has been assessed by conducting repeat-dose toxicity studies in rats and dogs that received four doses of Formulation H. Although it is recommended that the number of doses administered in systemic toxicity studies should exceed the number of doses administered in clinical studies (four doses) in accordance with the Guideline on Non-clinical Evaluation of Preventive Vaccines for Infectious Diseases (PFSB/ELD Notification No. 0527-1 dated May 27, 2010), PMDA considers that the applicant's explanation for the possibility of assessment of systemic toxicity of the DPT-sIPV vaccine, which Formulation H was administered at doses 10- to 50-fold higher than the proposed clinical doses in these repeat-dose toxicity studies, is acceptable.

3.(iii).B.(2) Cumulative irritation

When the cumulative irritation potential of the DPT-sIPV vaccine was evaluated, the dose per injection site was lower than the proposed clinical dose (0.5 mL). PMDA asked the applicant to explain the appropriateness of the evaluation.

The applicant responded as follows:

In a repeat-dose toxicity study in which rats received five injections of Formulation M at the same site, the dose per injection site was 0.109 to 0.346 mL/site. The observed local reactions were similar to those in rats that received a similar dose of DPT (0.107-0.354 mL/site). Although repeated injections at the same site were not studied, the dose of Formulation H per injection site was 0.5 to 10 mL/site in a repeat-dose toxicity study in beagle dogs. As a result, the incidence and severity of local reactions including foreign-body granulomas were dose-related, but tissue degeneration or necrosis did not occur even at 10 mL/site and no unpredecented local reaction was detected.

Based on the above, even when 0.5 mL/site of the DPT-sIPV vaccine is injected repeatedly at the same site, a new local reaction is unlikely to occur and the cumulative irritation of the candidate vaccine is considered similar to that of DPT. However, as the manufacturer, the applicant considers that it is important to investigate the cumulative irritation potential of 0.5 mL/site of the candidate vaccine. Thus, the results of an additional study will be reported by mid-November 2012.

PMDA accepted the applicant's explanation and the expert advisors commented that the applicant's explanation is understandable.

4. Clinical data

4.A Summary of the submitted data

As the efficacy and safety evaluation data, the results from 3 clinical studies presented in Table 4-1 were submitted.

Table 4-1. Summary of clinical studies

Phase	Study ID	Design	Study population	No. of subjects enrolled	Dose/Route of administration	Immunization schedule
I	BK-4SP /001	Single-blind	Healthy adult male subjects (20-35 years)	DPT-sIPV group: 17 Placebo group: 3	0.5 mL/ Subcutaneous	A single dose
II	BK-4SP /002	Randomized, double-blind	Healthy children (3 to <90 months of age)	Formulation H group: 39 Formulation M group: 41 Formulation L group: 39	0.5 mL/ Subcutaneous	Primary immunization: three doses at 3- to 8-week intervals Booster immunization: a single dose 6-12 months after the primary immunization
III	BK-4SP /003[a)]	Randomized, double-blind	Healthy children (3 to <74 months of age)	DPT-sIPV group: 247 (DPT-sIPV[a)]+OPV placebo) Control group: 125 (DPT[a)]+OPV)	0.5 mL/ Subcutaneous	・DPT-sIPV or DPT Primary immunization: three doses at 3- to 8-week intervals Booster immunization: a single dose 6-12 months after the primary immunization ・OPV or OPV placebo Two doses at least 6 weeks apart between study visit 4-7 weeks after the primary immunization with DPT-sIPV or DPT and up to 5 weeks prior to booster immunization

DPT: Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine, OPV: Live Oral Poliomyelitis Vaccine

a) Freeze-dried Haemophilus Type b Vaccine (Hib) only was allowed to be coadministered.

Table 4-2. Quantities of active ingredients in 0.5 mL of DPT-sIPV or DPT

Ingredient	DPT	Formulation H	Formulation M	Formulation L
Bordetella pertussis protective antigen	≥ 4 units	≥ 4 units		
Diphtheria toxoid	≤ 15 Lf	≤ 15 Lf		
Tetanus toxoid	≤ 2.5 Lf	≤ 2.5 Lf		
Inactivated poliovirus type 1	－	3 DU	1.5 DU	0.75 DU
Inactivated poliovirus type 2	－	100 DU	50 DU	25 DU
Inactivated poliovirus type 3	－	100 DU	50 DU	25 DU

－: Not contained

4.A.(1) Japanese phase I clinical study (5.3.5.1-1, Study BK-4SP/001; Study period, [redacted] 20[redacted] to [redacted] 20[redacted])

A placebo-controlled, single-blind study in healthy adult male subjects aged between 20 and 35 (Target sample size of 20; 17 subjects in the Formulation H group, 3 subjects in the placebo group) was conducted at a single center in Japan to evaluate the safety of Formulation H (Table 4-2) (DPT-sIPV group) vs. saline (placebo group). A single dose of 0.5 mL of Formulation H or saline was to be given by subcutaneous injection.

In the study, since adults with high diphtheria antibody titers may develop severe allergic reactions after vaccination, 20 enrolled subjects (17 subjects in the Formulation H group, 3 subjects in the placebo group) were confirmed to have no local reactions of induration of ≥15 mm in an intradermal test with the DPT vaccine 2 days prior to study vaccination. Then the subjects received study vaccine, and all of the subjects were included in the safety analysis population.

The percentage of subjects who had at least one adverse event in the 27- to 29-day period following study vaccination (starting from the following day of vaccination; the same rule applies hereinafter) was 88.2% (15 of 17 subjects) in the Formulation H group and 66.7% (2 of 3 subjects) in the placebo group and there were no deaths or serious adverse events. There were no abnormal changes in white blood cell count, platelet count, liver function tests and other laboratory parameters that are of safety concern. While no adverse reactions were reported in the placebo group, 82.4% of the Formulation H group (14 of 17 subjects) experienced at least one adverse reaction. Adverse reactions reported by at least 2 subjects in the Formulation H group were as shown in Table 4-3. In addition, ventricular extrasystoles occurred in 1 subject in the Formulation H group and its causal relationship to study vaccine could not be denied. However, since the event was considered peculiar to the subject, a follow-up was deemed unnecessary.

Table 4-3. Adverse reactions reported by at least 2 subjects in the Formulation H group (Safety analysis population)

Adverse reaction	Formulation H group (N = 17)	
	n	%
Injection site erythema	12	70.6
Injection site pain	7	41.2
Injection site swelling	5	29.4
Neutrophil count increased	4	23.5
Neutrophil percentage increased	4	23.5
Injection site induration	2	11.8
Injection site pruritus	2	11.8

N: No. of subjects included in the analysis population, n: No. of subjects with adverse reaction

4.A.(2) Japanese phase II clinical study (5.3.5.1-2, Study BK-4SP/002; Study period, [redacted] 20[redacted] to [redacted] 20[redacted])

A multicenter, randomized, double-blind, parallel-group, comparative study in healthy children 3 to <90 months of age (Target sample size of 105; 35 subjects per group) was conducted at 12 centers in Japan to evaluate the immunogenicity and safety of Formulation H, Formulation M, and Formulation L, i.e. three different antigen doses of the DPT-sIPV vaccine (Table 4-2).

Subjects were to receive a total of four doses of 0.5 mL of Formulation H, Formulation M, or Formulation L by subcutaneous injection: three doses at 3- to 8-week intervals (primary immunization) and a single dose 6 to 12 months after the third dose (booster immunization).

A total of 119 subjects (39 subjects in the Formulation H group, 41 subjects in the Formulation M group, 39 subjects in the Formulation L group) were enrolled into the study. All of the enrolled subjects were included in the safety analysis population and 118 subjects (39 subjects in the Formulation H group, 40 subjects in the Formulation M group, 39 subjects in the Formulation L group) were included in the Full Analysis Set (FAS), which was used for the primary analysis for immunogenicity. Excluded was 1 subject in the Formulation M group (no post-vaccination immunogenicity data due to consent withdrawal).

In the study, the blind was to be broken after the immunogenicity and safety data were frozen after the third dose (28-49 days after the third dose in Study BK-4SP/002) and subsequent assessments were to be performed in an unblinded manner. In order to assess the doses of inactivated poliovirus antigens to be used for a phase III clinical study, for immunogenicity evaluation, neutralizing antibody titers against attenuated strains of poliovirus types 1, 2, and 3 after the third dose of Formulation H, Formulation M, or Formulation L were measured to determine the following: (a) neutralizing antibody seropositivity rate (the percentage of subjects with neutralizing antibody titers of ≥1:8), (b) neutralizing antibody seroconversion rate (the percentage of subjects who converted from seronegative [antibody titer <1:8] before vaccination to seropositive after vaccination and subjects with a post-vaccination antibody titer ≥4-fold the pre-vaccination antibody titer), and (c) mean neutralizing antibody titer (log_2). In the FAS, the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the third dose were 100% (39 of 39 subjects) in all groups and the neutralizing antibody seroconversion rates were all 100% (39 of 39 subjects), except for 94.9% (37 of 39 subjects) in the Formulation M group and 89.7% (35 of 39 subjects) in the Formulation L group for serotype 1. The mean neutralizing antibody titers (log_2) after the third dose are shown in Table 4-4.

Table 4-4. Mean neutralizing antibody titers against attenuated strains of poliovirus (log_2)[a] after the third dose (FAS)

	Formulation H group (N = 39)	Formulation M group (N = 39)	Formulation L group (N = 39)
	Mean (SD)	Mean (SD)	Mean (SD)
Type 1	10.76 (1.56)	10.04 (1.69)	9.35 (1.85)
Type 2	10.90 (1.12)	10.19 (1.58)	9.45 (1.53)
Type 3	10.72 (1.31)	10.13 (1.47)	9.54 (1.26)

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) <3.0 were to be treated as 1.5.

Regarding safety, the incidence of adverse events in the observation period between the first dose and the study visit after the third dose or between the fourth dose and the study visit after the fourth dose (28-49 days after the fourth dose in Study BK-4SP/002) was 100% (39 of 39 subjects) in the Formulation H group, 100% (41 of 41 subjects) in the Formulation M group, and 100% (39 of 39 subjects) in the Formulation L group. The incidence of adverse reactions was 94.9% (37 of 39 subjects) in the Formulation H group, 80.5% (33 of 41 subjects) in the Formulation M group, and 89.7% (35 of 39 subjects) in the Formulation L group. Adverse events and/or adverse reactions that were reported by at least 10% of subjects in any group are shown in Table 4-5.

Table 4-5. Adverse events and/or adverse reactions that were reported by at least 10% of subjects in any group (Safety analysis population)

		Formulation H group (N = 39)				Formulation M group (N = 41)				Formulation L group (N = 39)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%	n	%	n	%
Local vaccination site reactions	Vaccination site erythema	27	69.2	27	69.2	29	70.7	29	70.7	32	82.1	32	82.1
	Vaccination site swelling	16	41.0	16	41.0	20	48.8	20	48.8	18	46.2	18	46.2
	Vaccination site induration	9	23.1	9	23.1	14	34.1	14	34.1	8	20.5	8	20.5
	Vaccination site haematoma	4	10.3	2	5.1	3	7.3	3	7.3	1	2.6	0	0
Systemic reactions	Nasopharyngitis	22	56.4	0	0	23	56.1	0	0	21	53.8	0	0
	Pyrexia	26	66.7	21	53.8	19	46.3	11	26.8	20	51.3	15	38.5
	Upper respiratory tract inflammation	19	48.7	3	7.7	18	43.9	1	2.4	22	56.4	4	10.3
	Rhinorrhoea	12	30.8	7	17.9	16	39.0	2	4.9	8	20.5	1	2.6
	Rash	9	23.1	2	5.1	12	29.3	3	7.3	16	41.0	3	7.7
	Diarrhoea	10	25.6	1	2.6	10	24.4	2	4.9	11	28.2	2	5.1
	Cough	8	20.5	3	7.7	7	17.1	2	4.9	8	20.5	1	2.6
	Gastroenteritis	10	25.6	0	0	7	17.1	1	2.4	4	10.3	0	0
	Eczema	3	7.7	1	2.6	6	14.6	1	2.4	6	15.4	1	2.6
	Pharyngitis	7	17.9	0	0	6	14.6	0	0	1	2.6	0	0
	Dermatitis diaper	6	15.4	0	0	6	14.6	0	0	8	20.5	0	0
	Vomiting	7	17.9	2	5.1	5	12.2	2	4.9	5	12.8	2	5.1
	Exanthema subitum	5	12.8	0	0	5	12.2	0	0	8	20.5	0	0
	Dry skin	2	5.1	0	0	5	12.2	0	0	4	10.3	0	0
	Otitis media	6	15.4	0	0	4	9.8	0	0	4	10.3	0	0
	Dermatitis contact	4	10.3	0	0	1	2.4	0	0	4	10.3	0	0

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction

Serious adverse events during the observation period included 4 events reported by 4 subjects in the Formulation H group (pneumonia mycoplasmal, respiratory syncytial virus bronchiolitis, gastroenteritis rotavirus, bronchitis), 2 events reported by 2 subjects in the Formulation M group (pseudocroup, exanthema subitum), and 5 events reported by 4 subjects in the Formulation L group (bronchopneumonia, otitis media, arthritis bacterial, anaphylactic reaction, Kawasaki's disease), but a causal relationship to study vaccine was denied for all events. There were no adverse events leading to study discontinuation or deaths.

4.A.(3) Japanese phase III clinical study (5.3.5.1-3, Study BK-4SP/003; Study period, [redacted] 20[redacted] to [redacted] 20[redacted])

A multicenter, randomized, double-blind, parallel-group, comparative study in healthy children 3 to <74 months of age (Target sample size of 326; 217 subjects in the DPT-sIPV group, 109 subjects in the control group) was conducted at 32 centers in Japan to evaluate the immunogenicity and safety of Formulation M (Table 4-2) plus an oral solution containing no attenuated strains of poliovirus (hereinafter "OPV placebo") (DPT-sIPV group) vs. DPT plus Live Oral Poliomyelitis Vaccine (OPV) (control group).

Subjects were to receive a total of four doses of 0.5 mL of DPT-sIPV or DPT by subcutaneous injection: three doses at 3- to 8-week intervals (primary immunization) and a single dose 6 to 12 months after the third dose (booster immunization). In addition, subjects were to receive two oral doses of 0.05 mL of OPV placebo or OPV, at least 6 weeks apart, between 4 to 7 weeks after the third dose of DPT-sIPV or DPT and up to 5 weeks prior to the fourth dose. Optional Freeze-dried Haemophilus Type b Vaccine (Hib) was allowed to be coadministered with DPT-sIPV or DPT.

A total of 372 subjects (247 subjects in the DPT-sIPV group, 125 subjects in the control group) were enrolled into the study. All of the enrolled subjects were included in the safety analysis population and 368 subjects (246 subjects in the DPT-sIPV group, 122 subjects in the control group) were included in the FAS, which was used for the primary analysis for immunogenicity. Excluded were 4 subjects without post-vaccination immunogenicity data (consent withdrawal [1 subject], moving out of the area [2 subjects], blood samples left at room temperature [1 subject]).

For immunogenicity evaluation, antibody titers were measured prior to the first dose of DPT-sIPV or DPT, after the third dose of DPT-sIPV or DPT (4-7 weeks after the third dose in Study BK-4SP/003), prior to the fourth dose of DPT-sIPV or DPT, and after the fourth dose of DPT-sIPV or DPT (4-7 weeks after the fourth dose in Study BK-4SP/003).

The primary endpoint was the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the third dose of DPT-sIPV. The seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the third dose in the DPT-sIPV group and their 95% confidence intervals (FAS) were all 100% (246 of 246 subjects) [98.5, 100] and the lower limit of the 95% confidence interval exceeded the pre-defined level of 90 (%) for all three serotypes.

On the other hand, the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus after the fourth dose in the control group (≥9 weeks after the second dose of OPV) and their 95% confidence intervals (FAS) were 100% (121 of 121 subjects) [97.0, 100] for serotypes 1 and 2 and 87.6% (106 of 121 subjects) [80.4, 92.9] for serotype 3.

The mean neutralizing antibody titers against attenuated strains of poliovirus types 1, 2, and 3 (log_2) over time in the DPT-sIPV and control groups are shown in Table 4-6.

Table 4-6. Mean neutralizing antibody titers against attenuated strains of poliovirus (log_2)[a] (FAS)

	Prior to the first dose[b]	After the third dose[b]	Prior to the fourth dose[c]	After the fourth dose[c]
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
DPT-sIPV group	N = 246	N = 246	N = 243	N = 243
Type 1	2.72 (1.73)	10.76 (1.83)	9.73 (2.33)	12.53 (1.42)
Type 2	2.96 (1.70)	10.95 (1.25)	9.93 (1.56)	13.39 (1.27)
Type 3	1.66 (0.82)	10.76 (1.52)	8.89 (1.86)	12.89 (1.33)
Control group	N = 121	N = 121	N = 120	N = 121
Type 1	2.76 (1.90)	2.13 (1.86)	12.27 (1.36)	12.03 (1.42)
Type 2	3.01 (1.88)	1.94 (1.55)	11.15 (1.39)	10.83 (1.38)
Type 3	1.66 (0.66)	1.51 (0.14)	7.80 (2.98)	7.55 (2.91)

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) <3.0 were to be treated as 1.5.
b) Prior to the first dose of OPV in the control group
c) After the second dose of OPV in the control group

The seropositivity rates for antibodies against *Bordetella pertussis* (pertussis toxin [PT] and filamentous hemagglutinin [FHA]), diphtheria toxin, and tetanus toxin after the third dose of DPT-sIPV or DPT (the percentages of subjects with positive antibody titers) and their 95% confidence intervals (FAS) were all 100% in the DPT-sIPV and control groups (246 of 246 subjects in the DPT-sIPV group, 122 of 122 subjects in the control group), except for 99.6% (245 of 246 subjects) [97.8, 100] for tetanus toxin in the DPT-sIPV group. Seropositivity was defined as ≥10 U/mL for anti-PT antibody; ≥10 U/mL for anti-FHA antibody; ≥0.1 international units (IU)/mL for anti-diphtheria toxin antibody; and ≥0.01 U/mL for anti-tetanus toxin antibody (synonymous with ≥0.01 IU/mL as an antibody titer is determined on the basis of the positive control assigned a value in IU).

The geometric mean antibody titers against different antigens over time in the DPT-sIPV and control groups are shown in Table 4-7.

Table 4-7. Geometric mean antibody titers against *Bordetella pertussis* (PT and FHA; U/mL), diphtheria toxin (IU/mL), and tetanus toxin (U/mL)[a] (FAS)

	Prior to the first dose	After the third dose	Prior to the fourth dose	After the fourth dose
	Geometric mean (Geometric standard deviation)	Geometric mean (Geometric standard deviation)	Geometric mean (Geometric standard deviation)	Geometric mean (Geometric standard deviation)
DPT-sIPV group	N = 246	N = 246	N = 244	N = 244
PT	0.79 (2.51)	203.56 (1.61)	83.36 (2.04)	212.50 (1.82)
FHA	2.62 (2.23)	92.29 (1.76)	44.75 (2.13)	179.35 (2.01)
Diphtheria	0.008 (2.561)	1.302 (2.394)	1.610 (3.392)	8.418 (2.041)
Tetanus	0.017 (4.007)	0.905 (2.947)	1.055 (6.151)	3.097 (3.136)
Control group	N = 121	N = 122	N = 120	N = 121
PT	0.91 (2.91)	187.65 (1.82)	78.08 (1.78)	185.54 (1.72)
FHA	3.02 (2.53)	120.21 (1.83)	54.24 (1.86)	208.32 (1.89)
Diphtheria	0.008 (2.563)	0.866 (2.459)	1.144 (3.151)	5.449 (2.131)
Tetanus	0.017 (3.747)	1.117 (2.510)	1.147 (4.306)	3.528 (3.102)

N: No. of subjects included in the analysis population

a) Anti-PT or anti-FHA antibody titers <0.1 U/mL were to be treated as 0.05 U/mL; Anti-diphtheria toxin antibody titers <0.01 IU/mL were to be treated as 0.005 IU/mL; Anti-tetanus toxin antibody titers <0.01 U/mL were to be treated as 0.005 U/mL.

Regarding safety, the observation period was the period between the first dose and the study visit after the third dose of DPT-sIPV or DPT or between the fourth dose and the study visit after the fourth dose or 5 weeks from each dose of OPV placebo or OPV. The incidence of adverse events was 99.6% (246 of 247 subjects) in the DPT-sIPV group and 100% (125 of 125 subjects) in the control group. The incidence of adverse reactions was 89.5% (221 of 247 subjects) in the DPT-sIPV group and 94.4% (118 of 125 subjects) in the control group. Adverse events and/or adverse reactions that were reported by at least 5% of subjects in either group are shown in Table 4-8.

Table 4-8. Adverse events and/or adverse reactions that were reported by at least 5% of subjects in either group
(Safety analysis population)

		DPT-sIPV group (N = 247)				Control group (N = 125)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%
Vaccination site[a)]	Vaccination site erythema	184	74.5	184	74.5	108	86.4	108	86.4
	Vaccination site induration	148	59.9	148	59.9	89	71.2	89	71.2
	Vaccination site swelling	99	40.1	99	40.1	75	60.0	75	60.0
Others	Pyrexia	235	95.1	112	45.3	110	88.0	58	46.4
	Nasopharyngitis	138	55.9	6	2.4	64	51.2	3	2.4
	Diarrhoea	113	45.7	55	22.3	50	40.0	28	22.4
	Rhinorrhoea	98	39.7	18	7.3	56	44.8	10	8.0
	Upper respiratory tract inflammation	97	39.3	3	1.2	50	40.0	3	2.4
	Dermatitis diaper	75	30.4	0	0	31	24.8	0	0
	Rash	71	28.7	20	8.1	31	24.8	14	11.2
	Gastroenteritis	66	26.7	6	2.4	27	21.6	3	2.4
	Cough	63	25.5	14	5.7	28	22.4	8	6.4
	Exanthema subitum	58	23.5	0	0	24	19.2	0	0
	Vomiting	41	16.6	14	5.7	20	16.0	9	7.2
	Upper respiratory tract infection	36	14.6	0	0	10	8.0	0	0
	Otitis media	29	11.7	0	0	17	13.6	0	0
	Heat rash	28	11.3	0	0	13	10.4	0	0
	Dermatitis contact	28	11.3	0	0	9	7.2	0	0
	Bronchitis	25	10.1	1	0.4	12	9.6	0	0
	Conjunctivitis	25	10.1	0	0	6	4.8	0	0
	Hand-foot-and-mouth disease	23	9.3	0	0	5	4.0	0	0
	Eczema	21	8.5	1	0.4	10	8.0	1	0.8
	Arthropod sting	17	6.9	0	0	9	7.2	0	0
	Eczema infantile	15	6.1	0	0	2	1.6	1	0.8
	Dry skin	14	5.7	0	0	6	4.8	0	0
	Urticaria	13	5.3	3	1.2	11	8.8	3	2.4
	Varicella	13	5.3	0	0	4	3.2	0	0
	Influenza	4	1.6	0	0	8	6.4	0	0

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction
a) Events at the injection site for Hib vaccine are not included.

Serious adverse events during the observation period included 17 events reported by 15 subjects in the DPT-sIPV group (bronchitis [3], pneumonia [2], respiratory syncytial virus bronchiolitis [2], bronchopneumonia, gastroenteritis, gastroenteritis rotavirus, gastroenteritis viral, otitis media, pneumonia respiratory syncytial viral, Kawasaki's disease, upper respiratory tract inflammation, inguinal hernia, vesicoureteric reflux) and 6 events reported by 6 subjects in the control group (gastroenteritis rotavirus, respiratory syncytial virus bronchitis, febrile convulsion, asthma, intussusception, pyrexia), of which pyrexia occurring 4 days after the second dose in the control group was classified as a serious adverse reaction because its causal relationship to study vaccine could not be denied. There were no adverse events leading to study discontinuation or deaths.

4.B Outline of the review by PMDA

4.B.(1) Clinical data package

The applicant explained the data comprising the clinical data package as follows:

DPT-sIPV is a quadruple vaccine consisting of a combination of the bulk of the approved DPT vaccine and inactivated poliovirus. It was decided to evaluate the efficacy and safety of DPT-sIPV for the proposed indication of the prevention of pertussis, diphtheria, tetanus, and acute poliomyelitis based on three clinical studies: Study BK-4SP/001, Study BK-4SP/002, and Study BK-4SP/003.

It was considered possible to determine the quantities of the *Bordetella pertussis* protective antigen, diphtheria toxoid, and tetanus toxoid among the active ingredients of the DPT-sIPV vaccine, based on those of the approved DPT vaccine, which has been widely used in children in Japan and whose efficacy and safety have been confirmed. On the other hand, the doses of inactivated poliovirus antigens were determined by studying the following three formulations: Formulation H, which showed equivalent immunogenicity to two different inactivated polio vaccines derived from virulent strains of poliovirus (vIPV) that have been approved overseas in non-clinical studies; Formulation M with half the inactivated poliovirus antigen contents of Formulation H; and Formulation L with a quarter of the inactivated poliovirus antigen contents of Formulation H (Table 4-2) [see "3.(i).A.(1).3) Inactivated poliovirus immunogenicity studies in cynomolgus monkeys"]. After the tolerability of Formulation H was demonstrated in Study BK-4SP/001, Formulation M was selected based on a dose-finding study (Study BK-4SP/002). It was decided to conduct a confirmatory study (Study BK-4SP/003) using Formulation M to evaluate its immunogenicity by poliovirus neutralizing antibody response. Immunogenicity evaluation by neutralizing antibody response was considered appropriate because it is difficult to assess the protective efficacy against polio in Japan where wild-type polio has been eradicated and neutralizing antibody in blood is considered protective against polio (*Preventive Vaccine Committee, Infection Department, Health Sciences Council, Fact sheet on poliomyelitis vaccine,* National Institute of Infectious Diseases, July 7, 2010, Plotkin. *Vaccines.* 5th ed. Saunders; 2008: 605-629). Study BK-4SP/003 also evaluated the safety of DPT-sIPV and the immunogenicity of the DPT components of DPT-sIPV compared with the control group. Study BK-4SP/003 did not compare the immunogenicity of DPT-sIPV with that of a comparator, OPV, because direct comparison of neutralizing antibodies in blood only was considered of little clinical significance taking account of the differences in the immune response due to disparities in the characteristics of the formulation, method of administration, and the immunization schedule.

PMDA considers as follows:

Given that DPT-sIPV is a vaccine product that will replace the approved DPT and OPV vaccines, essentially, the non-inferiority of DPT-sIPV to DPT plus OPV (control group) in terms of the immunogenicity of each active ingredient should have been tested. However, the applicant thought that simple comparison of the measurements of neutralizing antibodies in blood between IPV and OPV is of little significance due to differences in the immunization schedule and mode of action, which is understandable. Also regarding the immunogenicity of the DPT components of the DPT-sIPV vaccine, which are the same as those of the approved DPT vaccine, though non-inferiority was not tested, it was eventually found that there were no major differences in the seropositivity rates or geometric mean antibody titers between the DPT-sIPV vaccine and the approved DPT vaccine [see "4.B.(2).3) Efficacy against pertussis, diphtheria, and tetanus"]. Therefore, it is possible to evaluate the immunogenicity and safety of DPT-sIPV based on the clinical data package proposed by the applicant.

4.B.(2) Efficacy

4.B.(2).1) Selection of the primary endpoint

The applicant explained the rationale for selecting the primary endpoint for Study BK-4SP/003 as follows:

The efficacy of the novel inactivated poliovirus component of the DPT-sIPV vaccine should be evaluated by the percentage of subjects with clinically significant neutralizing antibody titers after the primary immunization with DPT-sIPV (after the third dose). Clinically significant neutralizing antibody titers should be defined as titers of ≥1:8 for the following two reasons:

· It has been reported from an US large trial with vIPV that a neutralizing antibody titer of ≥1:4 was sufficient to protect against polio (*Evaluation of the 1954 field trial of poliomyelitis vaccine: final report*. 1957).

· In the clinical development of vIPV that has been approved overseas and combination vaccines containing vIPV, a more stringent criterion of a titer of ≥1:8 was employed widely (*Bull World Health Organ*, 74:253-268, 1996, Plotkin. *Vaccines.* 5th ed. Saunders; 2008: 605-629).

Based on the above, the neutralizing antibody seropositivity rates defined as the percentages of subjects with neutralizing antibody titers of ≥1:8 against attenuated strains of poliovirus types 1, 2, and 3 after the third dose of DPT-sIPV have been chosen as the primary endpoint for Study BK-4SP/003.

PMDA reviewed other publications (National Institute of Health Research Associate ed. *Vaccine Handbook*. 1994: 120-129, *J Infect Dis.* 2012;205:237-243, *N Engl J Med.* 2007;356:1536-1544,

Manual for the virological investigation of polio, WHO/EPI/GEN/97.01, WHO, 1997) as well as the applicant's explanation. As a result, PMDA considers that choosing the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the third dose of DPT-sIPV as the primary endpoint is appropriate.

4.B.(2).2) Efficacy against polio

Since the DPT-sIPV vaccine is the world's first vaccine containing inactivated polioviruses derived from attenuated strains as active ingredients, PMDA asked the applicant to discuss the immunogenicity of DPT-sIPV against wild-type or virulent strains of poliovirus in terms of protection against polio, as well as the immunogenicity of DPT-sIPV against attenuated strains of poliovirus.

The applicant responded as follows:

The basic reproductive number (R_0: the mean number of expected secondary infections resulting from a single infectious case) as a measure of the transmissibility of a human to human transmitted pathogen is 5 to 7 for poliovirus and the estimated herd immunity threshold needed to avoid epidemic for polio (R_0 is the basic reproductive number; $(1-1/R_0) \times 100$; hereinafter, this value is referred to as "herd immunity threshold") is 80% to 86% (*Epidemiol Rev*. 1993;15:265-302.). It has also been reported that the herd immunity threshold in advanced countries where polio epidemics seldom occur is 66% to 80% (Plotkin, *Vaccines*, 5th ed. Saunders; 2008: 631-685). Interpreting the above information conservatively, it was decided to use a threshold value of 90% for the primary endpoint of the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the third dose of DPT-sIPV for Study BK-4SP/003.

In Study BK-4SP/003, the neutralizing antibody seropositivity rate after the third dose of DPT-sIPV and its 95% confidence interval were 100% [98.5, 100] for all three serotypes [see "4.A.(3) Japanese phase III clinical study"]. As the lower limit of the 95% confidence interval for the neutralizing antibody seropositivity rate exceeded the pre-defined level of 90% for all serotypes, it was concluded that the efficacy of DPT-sIPV against polio was confirmed. The mean neutralizing antibody titers against attenuated strains of poliovirus over time (Table 4-6) showed that the fourth dose of DPT-sIPV induced a booster response (about 7-fold, about 11-fold, and about 16-fold the antibody titers prior to the fourth dose for serotypes 1, 2, and 3, respectively). Though simple comparison is impossible due to differences in the mode of action and the timing of sampling for antibody titer measurements, the mean neutralizing antibody titers after the fourth dose of DPT-sIPV were higher than those after the second dose of OPV

(prior to and after the fourth dose of the comparator) (Table 4-6).

Furthermore, crossreactivity to virulent strains of poliovirus used in the production of a foreign-approved vIPV vaccine (type 1, Mahoney strain; type 2, MEF-1 strain; type 3, Saukett strain), though the strain is different from wild-type strains of poliovirus that are circulating in some countries, was tested in Study BK-4SP/003 (Table 4-9).

Table 4-9. Mean neutralizing antibody titers against attenuated and virulent strains of poliovirus (log_2)[a] after vaccination with DPT-sIPV (Study BK-4SP/003)

	Attenuated strains (used in the production of DPT-sIPV)		Virulent strains (used in the production of foreign vIPV)	
	After the third dose N = 246	After the fourth dose N = 243	After the third dose N = 246	After the fourth dose N = 54[b]
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Type 1	10.76 (1.83)	12.53 (1.42)	6.65 (1.63)	8.72 (1.60)
Type 2	10.95 (1.25)	13.39 (1.27)	9.66 (1.48)	12.62 (1.37)
Type 3	10.76 (1.52)	12.89 (1.33)	9.37 (1.65)	12.08 (1.51)

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) <3.0 were to be treated as 1.5.
b) Specimens of a target of 50 subjects were randomly selected from serum samples to measure neutralizing antibody titers.

The mean neutralizing antibody titers against virulent strains of poliovirus tended to be generally lower than those against attenuated strains of poliovirus and were especially low for serotype 1, but all values were greater than 3 (log_2), which was equivalent to a neutralizing antibody titer of 1:8. The seropositivity rates for neutralizing antibodies against virulent strains of poliovirus after the third dose of DPT-sIPV and their 95% confidence intervals were 98.8% (243 of 246 subjects) [96.5, 99.7] for serotype 1 and 100% (246 of 246 subjects) [98.5, 100] for serotypes 2 and 3 and 3 of the 246 subjects were seronegative for serotype 1 after the third dose. All of the three seronegative subjects were found to be seropositive (≥ 3) with neutralizing antibody titers (log_2) of 8.0, 8.5, and 6.5, respectively, after the fourth dose.

Since the above finding suggested that the crossreactivity of antibodies elicited by DPT-sIPV against virulent strains of poliovirus, the efficacy of DPT-sIPV against polio can be expected.

Based on the applicant's explanation, PMDA considers that the immunogenicity of DPT-sIPV against attenuated and virulent strains of poliovirus and a booster response after the fourth dose of DPT-sIPV can be expected. Also, in an area where wild-type polio is prevalent, the protective efficacy of a combination vaccine containing vIPV against polio was assessed and 6 months after the second dose of the vaccine, 80% to 90% of subjects had neutralizing antibody titers of ≥1:4 against virulent strains of poliovirus types 1, 2, and 3 (*Rev Infect Dis*. 1984;6:S463-S466.). It has also been reported that the protective efficacy of two doses of vaccine and its 95% confidence interval were 89% [62, 97] (*Lancet.* 1988;331:897-899). Taking account of these reports etc., PMDA concluded that the efficacy of DPT-sIPV against polio can be expected.

4.B.(2).3) Efficacy against pertussis, diphtheria, and tetanus

The applicant explained the efficacy of DPT-sIPV against pertussis, diphtheria, and tetanus as follows:

In Study BK-4SP/003, the cut-off values for seropositivity for anti-diphtheria toxin and anti-tetanus toxin antibodies were defined as 0.1 IU/mL and 0.01 U/mL (synonymous with 0.01 IU/mL as an antibody titer is determined on the basis of the positive control assigned a value in IU), respectively, which were the seroprotective levels specified by NIID (*Report on National Epidemiological Surveillance of Vaccine-Preventable Diseases, 2003*, Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, MHLW, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, December 2004) and the cut-off values for seropositivity for anti-PT and anti-FHA antibodies were defined as 10 U/mL (equivalent to 10 ELISA units/mL in the literature; *Journal of Pediatric Practice.* 1990; 53:2275-2281.), which were the seroprotective levels estimated from convalescent antibody titers in children with whooping cough. The seropositivity rates for antibodies against PT, FHA, diphtheria toxin, and tetanus toxin after the third dose of DPT-sIPV or DPT are shown in Table 4-10. The difference in the seropositivity rate between the DPT-sIPV and control groups and its 95% confidence interval were −0.4 [−2.3, 2.7] for tetanus toxin and 0.0 [−1.5, 3.1] for others and there were no major differences in the seropositivity rates between the two groups. In addition, the geometric mean antibody titer against each antigen was variable, but the geometric mean antibody titers over time were similar between the two groups (Table 4-7).

Based on the above, the efficacy of DPT-sIPV against pertussis, diphtheria, and tetanus is comparable to that of the approved DPT vaccine and the immunogenicity of DPT-sIPV against each antigen can be expected.

Table 4-10. Seropositivity rates for antibodies against Bordetella pertussis, diphtheria toxin, and tetanus toxin after the third dose of DPT-sIPV (Study BK-4SP/003, FAS)

	DPT-sIPV group		Control group	
	n/N	% [95% CI]	n/N	% [95% CI]
PT	246/246	100 [98.5, 100]	122/122	100 [97.0, 100]
FHA	246/246	100 [98.5, 100]	122/122	100 [97.0, 100]
Diphtheria	246/246	100 [98.5, 100]	122/122	100 [97.0, 100]
Tetanus	245/246	99.6 [97.8, 100]	122/122	100 [97.0, 100]

N: No. of subjects included in the analysis population, n: No. of seropositive subjects

PMDA considers as follows:

WHO also has recommended the same cut-off values for seropositivity for antibodies against diphtheria toxin and tetanus toxin (*Wkly Epidemiol Rec*. 2006; 81:21-32, *Wkly Epidemiol Rec*. 2006; 81:197-208). On the other hand, the clinical significance of the cut-off values for seropositivity for antibodies against PT and FHA is unclear in some aspects. However, as there

were no major differences in the seropositivity rates for antibodies against diphtheria toxin and tetanus toxin between DPT-sIPV and DPT that has already been marketed and there were also no major differences in the geometric mean antibody titer against each antigen between the two vaccines, the applicant's view that the efficacy of DPT-sIPV against pertussis, diphtheria, and tetanus is comparable to that of the approved DPT vaccine is acceptable.

Based on the above, PMDA considers as follows:
The efficacy of DPT-sIPV against polio, pertussis, diphtheria, and tetanus can be expected. In the US, Europe, etc. where IPV or combination vaccines containing IPV have been introduced, an additional dose of IPV is given to children 4 to 6 years of age, before school entry. It is recommended to continue to assess the need for an additional dose of IPV in DPT-sIPV recipients also in Japan.

4.B.(3) Safety

As a result of the following reviews, PMDA concluded that there are no major differences in safety between the DPT-sIPV vaccine and the approved DPT vaccine, and DPT-sIPV is tolerable. However, as the submitted evaluation data include a limited number of subjects, PMDA considers that it is necessary to continue to collect safety information carefully via post-marketing surveillance etc.

4.B.(3).1) Comparison of safety

The applicant explained the safety of DPT-sIPV as follows:
The safety of DPT-sIPV vs. DPT was assessed by the analysis of events occurring, in Study BK-4SP/003, during the post-vaccination periods, between the first dose and the study visit after the third dose of DPT-sIPV or DPT, and between the fourth dose and the study visit after the fourth dose. The analysis excluded events occurring after vaccination with OPV placebo or OPV. The incidences of adverse events reported at a ≥5% higher incidence in the DPT-sIPV group than in the control group (pyrexia, diarrhoea, upper respiratory tract infection) and adverse reactions reported at higher incidences in the DPT-sIPV group than in the control group (diarrhoea, rhinorrhoea, cough) by maximum intensity are as shown in Table 4-11. There were no major differences in the incidence of adverse events or adverse reactions of severe (Grade 3 or 4) pyrexia or diarrhoea between the DPT-sIPV and control groups and severe upper respiratory tract infection, rhinorrhoea, or cough was not observed in either group. Therefore, the tolerability of DPT-sIPV is considered comparable to that of the approved DPT vaccine.

Table 4-11. Incidence by maximum intensity[a] (Study BK-4SP/003, Safety analysis population)

		DPT-sIPV group (N = 247)				Control group (N = 125)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%
Pyrexia[b]	Overall	220	89.1	96	38.9	103	82.4	51	40.8
	Grade 3	95	38.5	9	3.6	46	36.8	5	4.0
	Grade 4	2	0.8	0	0	1	0.8	0	0
Diarrhoea[c]	Overall	82	33.2	24	9.7	28	22.4	9	7.2
	Grade 3	4	1.6	0	0	1	0.8	0	0
	Grade 4	0	0	0	0	0	0	0	0
Upper respiratory tract infection[d]	Overall	31	12.6	0	0	6	4.8	0	0
	Grade 3	0	0	0	0	0	0	0	0
	Grade 4	0	0	0	0	0	0	0	0
Rhinorrhoea[e]	Overall	71	28.7	14	5.7	40	32.0	7	5.6
	Grade 3	0	0	0	0	0	0	0	0
	Grade 4	0	0	0	0	0	0	0	0
Cough[f]	Overall	56	22.7	13	5.3	23	18.4	6	4.8
	Grade 3	0	0	0	0	0	0	0	0
	Grade 4	0	0	0	0	0	0	0	0

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction
a) If more than one event occurred in the same subject, the maximum intensity was counted.
b) Grade 3: ≥39.0°C, persisted for ≤3 days; Grade 4: ≥39.0°C, persisted for ≥4 days
c) Grade 3: Frequent bowel movements, ≥9 times/day; Grade 4: Life-threatening
d) Grade 3: Requiring hospitalization and/or invasive treatment etc.; Grade 4: Requiring intensive and/or emergency treatment etc.
e) Grade 3: Runny nose almost all day preventing normal daily activities; Grade 4: No definition
f) Grade 3: Severe cough preventing normal daily activities and requiring hospitalization etc.; Grade 4: Life-threatening

PMDA considers as follows:

The applicant's explanation that the tolerability of DPT-sIPV is comparable to that of the approved DPT vaccine is acceptable.

No deaths occurred in any of the clinical studies included in the evaluation data, and serious adverse events reported in Studies BK-4SP/002 and BK-4SP/003 involving infants (the intended population for the vaccine) were diseases commonly seen in infants and causal relationship to DPT-sIPV was denied for all serious adverse events. In Study BK-4SP/002, as serious adverse events occurring outside the observation period (the period between the study visit after the third dose and the fourth dose), febrile convulsion (1 subject) and convulsion (1 subject) in the Formulation H group and febrile convulsion (1 subject) in the Formulation L group were reported, but their causal relationship to study vaccine was denied.

Based on the above, there were no particular serious adverse events of concern in the clinical studies and PMDA concluded that the safety profile of DPT-sIPV is tolerable.

4.B.(3).2) Clinically significant adverse reactions

"Shock, anaphylactoid reaction, acute thrombocytopenic purpura, encephalopathy, and convulsion" have been spontaneously reported with the approved DPT vaccine though the

incidences are unknown. As these events are likely to occur also with DPT-sIPV, the applicant will provide a caution in the package insert.

PMDA understands that these events are very rare and it is difficult to determine the accurate incidences of the events. However, since there is limited information on safety after vaccination with DPT-sIPV, PMDA considers that it is necessary to continue to collect information after the market launch.

4.B.(4) Clinical positioning and indication

The applicant explained the clinical positioning of the DPT-sIPV vaccine as follows:

In Japan where there are currently no reported cases of infection with wild-type polio, vaccine-associated paralytic poliomyelitis (VAPP) caused by OPV has been a problem (*Clinical Virology*. 1996; 24:162-169). With respect to widely used vIPV derived from virulent strains of poliovirus, a small-scale polio epidemic caused by virulent strains used in the production of vIPV has occurred in India (*Wkly Epidemiol Rec*. 2003;78:284.) and hence virus containment during the production of vIPV is thought to be an important issue. Taking account of this situation, WHO has also recommended the development and introduction of IPV derived from attenuated strains of poliovirus (*New polio vaccines for the post-eradication era*, WHO/V&B/00.20, WHO, 2000, *Global Polio Eradication Initiative Strategic Plan 2004-2008*, WHO, 2003). IPV derived from attenuated strains of poliovirus in the DPT-sIPV vaccine will be the first of its kind. Moreover, the Subcommittee on Polio and Measles Vaccines, Infection Committee, Infection Department, the 7th Health Sciences Council (in 2003) has recommended the introduction of a combined vaccine of DPT and IPV to increase the vaccination rate. The DPT-sIPV vaccine containing DPT and IPV derived from attenuated strains of poliovirus as active ingredients has no theoretical risk of VAPP and can provide priming against pertussis, diphtheria, tetanus, and polio simultaneously.

PMDA's view on the clinical positioning of the DPT-sIPV vaccine is as follows:

Based on the results of evaluation of the immunogenicity of DPT-sIPV against attenuated and virulent strains of poliovirus, the protective efficacy of DPT-sIPV against polio can be expected. The protective efficacy of DPT-sIPV against pertussis, diphtheria, and tetanus can also be expected and its safety profile is also tolerable. Thus, the DPT-sIPV vaccine can possibly replace DPT and OPV vaccines in clinical practice.

As a result of its review in "4.B.(2) Efficacy," PMDA concluded that the indication for the DPT-sIPV vaccine should be "the prevention of pertussis, diphtheria, tetanus, and acute

poliomyelitis."

4.B.(5) Dosage and administration

4.B.(5).1) Doses of antigens

The applicant explained the appropriateness of the doses of antigens in the DPT-sIPV vaccine as follows:

As described in "4.B.(1) Clinical data package," the doses of the DPT components of the DPT-sIPV vaccine were selected based on those found in the approved DPT vaccine and it was decided to select the doses of inactivated poliovirus antigens from among Formulation H, which showed comparable immunogenicity to vIPV that has been approved overseas in non-clinical studies, Formulation M with half the inactivated poliovirus antigen contents of Formulation H, and Formulation L with a quarter of the inactivated poliovirus antigen contents of Formulation H (Table 4-2).

Concerning immunogenicity, the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 (the percentages of subjects with neutralizing antibody titers of ≥1:8) after the primary immunization (after the third dose) in a dose-finding study BK-4SP/002 were 100% in all groups [see "4.A.(2) Japanese phase II clinical study"]. The neutralizing antibody seroconversion rate (the percentage of subjects who converted from seronegative [antibody titer <1:8] before vaccination to seropositive after vaccination and subjects who had a post-vaccination antibody titer ≥4-fold the pre-vaccination antibody titer) was 100% (39 of 39 subjects) in the Formulation H group for all serotypes, 94.9% (37 of 39 subjects) for serotype 1 and 100% (39 of 39 subjects) for serotypes 2 and 3 in the Formulation M group, and 89.7% (35 of 39 subjects) for serotype 1 and 100% (39 of 39 subjects) for serotypes 2 and 3 in the Formulation L group and the mean neutralizing antibody titers showed tendency of dose-dependence (Table 4-4). The criterion for seropositivity (a neutralizing antibody titer of ≥1:8) was not met in 1 subject each for serotypes 1, 2, and 3 in the Formulation L group prior to the fourth dose.

Regarding safety, in Study BK-4SP/002, there were no major differences in the incidence of adverse events or adverse reactions occurring in the period between the first dose and the study visit after the third dose among the different dose groups (Table 4-12) while the incidence of adverse reactions of pyrexia was higher in the Formulation H group than in the Formulation L and M groups (Table 4-13). When adverse events/adverse reactions occurring in the period between the fourth dose and the study visit after the fourth dose were included in the analysis, the incidence of adverse reactions was higher in the Formulation H group, the incidence of

adverse events or adverse reactions of pyrexia was also higher in the Formulation H group than in the Formulation M and L groups, and the incidence of adverse events or adverse reactions of Grade 3 or 4 pyrexia was also higher. On the other hand, adverse events with a higher incidence in the Formulation M group than in the Formulation H or L group, e.g. vaccination site induration, vaccination site swelling, and rhinorrhoea (Table 4-5) were mild in severity (Grade 1 or 2).

Table 4-12. Incidence of adverse events or adverse reactions (Study BK-4SP/002, Safety analysis population)

	Adverse event						Adverse reaction					
	Formulation H group		Formulation M group		Formulation L group		Formulation H group		Formulation M group		Formulation L group	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
After the first three doses	38/39	97.4	41/41	100	39/39	100	33/39	84.6	33/41	80.5	34/39	87.2
After four doses	39/39	100	41/41	100	39/39	100	37/39	94.9	33/41	80.5	35/39	89.7

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction

Table 4-13. Incidence of pyrexia by maximum intensity[a)] (Study BK-4SP/002, Safety analysis population)

	Adverse event						Adverse reaction					
	Formulation H group		Formulation M group		Formulation L group		Formulation H group		Formulation M group		Formulation L group	
	n	%	n	%	n	%	n	%	n	%	n	%
After the first three doses	N = 39		N = 41		N = 39		N = 39		N = 41		N = 39	
Overall	19	48.7	16	39.0	14	35.9	15	38.5	7	17.1	9	23.1
Grade 3[b)]	2	5.1	1	2.4	2	5.1	2	5.1	0	0	1	2.6
Grade 4[c)]	0	0	0	0	0	0	0	0	0	0	0	0
After four doses	N = 39		N = 41		N = 39		N = 39		N = 41		N = 39	
Overall	26	66.7	19	46.3	20	51.3	21	53.8	11	26.8	15	38.5
Grade 3[b)]	7	17.9	1	2.4	2	5.1	7	17.9	0	0	1	2.6
Grade 4[c)]	0	0	0	0	0	0	0	0	0	0	0	0

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction
a) If more than one event occurred in the same subject, the maximum intensity was counted.
b) ≥39.0°C, persisted for ≤3 days
c) ≥39.0°C, persisted for ≥4 days

Taking account of the immunogenicity and safety results from Study BK-4SP/002, a confirmatory study (Study BK-4SP/003) was conducted using Formulation M, which confirmed the immunogenicity and safety of the inactivated poliovirus and DPT components of the DPT-sIPV vaccine [see "4.B.(2) Efficacy" and "4.B.(3) Safety"].

Based on the above, it was concluded that the appropriate quantities of the active ingredients per 0.5 mL dose of the DPT-sIPV vaccine are ≤15 Lf for diphtheria toxoid, ≤2.5 Lf for tetanus toxoid, ≥4 units for the *Bordetella pertussis* protective antigen, 1.5 DU for inactivated poliovirus type 1, 50 DU for inactivated poliovirus type 2, and 50 DU for inactivated poliovirus type 3.

PMDA accepted the applicant's explanation.

4.B.(5).2) Immunization schedule

The applicant explained the rationale for the proposed immunization schedule as follows:

Taking into account that the DPT-sIPV vaccine will replace the approved DPT vaccine and referring to the DPT-IPV immunization schedule in the US/Europe, the immunization schedule for Studies BK-4SP/002 and BK-4SP/003 was determined as follows: three primary series doses of DPT-sIPV were to be given at 3- to 8-week intervals and a booster dose of DPT-sIPV was to be given 6 to 12 months after the primary immunization. The number of vaccinated subjects by dosing interval for primary immunization in Study BK-4SP/003 is shown in Table 4-14. In this study, the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the primary immunization were 100% and there were no differences in the mean neutralizing antibody titers by the interval between doses. The booster response was not influenced by differences in the interval between the primary immunization and booster (6-12 months).

Based on the above study results, and taking also into account that the DPT-sIPV vaccine will replace the approved DPT vaccine, the DPT-sIPV immunization schedule can be determined as follows: the interval between the primary series doses is 3 to 8 weeks and the interval between the primary immunization and booster is ≥6 months (normally 12-18 months after the completion of primary immunization).

Table 4-14. Number of vaccinated subjects by dosing interval for primary immunization in Study BK-4SP/003

Dosing interval	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
Dose 1 to 2	72	122	44	3	5	—
Dose 2 to 3	85	109	39	8	1	4

In the US where vIPV has been introduced, it is recommended that the minimum interval between the primary series doses of IPV (two doses) should be 4 weeks, since shorter intervals between doses may lead to lower seroconversion rates (*The Pinkbook.* 12th ed. 2011: chapter 17 Poliomyelitis). Although there is little information on the immunogenicity of the inactivated poliovirus component derived from attenuated strains, based on the results of Study BK-4SP/003, PMDA concluded that the expected immune response to all active ingredients of the DPT-sIPV vaccine can be obtained when the primary series doses of DPT-sIPV are given at intervals of at least 3 weeks.

With respect to the interval between the primary immunization and booster, a booster response was observed when a booster dose was given ≥6 months after the primary immunization in Study BK-4SP/003. It is reported that a minimum interval of 6 months is important for the

efficacy of vIPV as well (*The Pinkbook.* 12th ed. 2011: chapter 17 Poliomyelitis.), and an interval of ≥6 months is recommended for the approved DPT vaccine as well. Taking account of these points, PMDA considers that the appropriate interval between the primary immunization and booster should be ≥6 months for DPT-sIPV.

4.B.(5).3) Intended population for vaccine

PMDA considers as follows:

Since the DPT-sIPV vaccine was developed as a vaccine product that would replace the DPT vaccine, which is given to children 3 to 90 months of age in accordance with the Order for Enforcement of the Preventive Vaccinations Act, Study BK-4SP/002 involving children 3 to <90 months of age and Study BK-4SP/003 involving children 3 to <74 months of age were conducted, and those studies have confirmed the efficacy and safety of DPT-sIPV. In addition, as an additional dose of diphtheria toxoid may cause allergic reactions in persons previously primed with DPT during their infancy (National Institute of Health Research Associate ed. *Vaccine Handbook.*), DPT-sIPV should be used for primary and booster immunization of children only.

As a result of the above reviews, PMDA concluded that the appropriate dosage and administration statement for the DPT-sIPV vaccine should be as shown below and the interval between doses should be described in the Precautions of Dosage and Administration section of the package insert etc. as well, which will be discussed at the Expert Discussion.

[Dosage and administration]

Primary immunization: The usual primary series for children consist of three doses of 0.5 mL each given by subcutaneous injection at intervals of at least 3 weeks.

Booster immunization: The usual booster dose for children is a single 0.5 mL dose given by subcutaneous injection at least 6 months after the primary immunization.

4.B.(6) Concomitant use with other vaccines

The applicant explained concomitant administration of the DPT-sIPV vaccine with other vaccines as follows:

DPT-sIPV is likely to be coadministered with Freeze-dried Haemophilus Type b Vaccine (Hib) and heptavalent pneumococcal conjugate vaccine (PCV7), which are subject to the urgent project for promotion of vaccination, administered as a series of three doses for primary immunization, and started normally at 2 to <7 months of age. Because Hib was allowed to be

coadministered with DPT-sIPV in Study BK-4SP/003, the immunogenicity and safety of DPT-sIPV alone or with Hib were evaluated. As shown in Table 4-15 and Table 4-16, coadministration with Hib did not significantly affect the immunogenicity or safety of DPT-sIPV.

In a post-marketing clinical study of Hib that has been approved in Japan, there were no immunogenicity or safety problems with coadministration with DPT. It has also been reported at scientific meetings that according to a specified use-results survey for PCV7 that has been approved in Japan, there are no noteworthy safety issues concerning coadministration with Hib or DPT etc. (*Program/Abstracts for the 14th Annual Meeting of the Japanese Society for Vaccinology*, 57, 2010, *Program/Abstracts for the 15th Annual Meeting of the Japanese Society for Vaccinology*, 96, 2011).

Table 4-15. Mean neutralizing antibody titers against attenuated strains of poliovirus (log_2)[a)] in subjects who received DPT-sIPV with or without Hib (Study BK-4SP/003, FAS)

	Prior to the first dose	After the third dose	Prior to the fourth dose	After the fourth dose
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
DPT-sIPV	N = 136	N = 136	N = 235	N = 235
Type 1	2.70 (1.81)	10.82 (1.79)	9.73 (2.34)	12.52 (1.43)
Type 2	2.85 (1.77)	10.97 (1.33)	9.97 (1.56)	13.39 (1.29)
Type 3	1.65 (0.81)	10.84 (1.40)	8.94 (1.85)	12.88 (1.34)
DPT-sIPV + Hib	N = 110[b)]	N = 110[b)]	N = 8[c)]	N = 8[c)]
Type 1	2.75 (1.64)	10.69 (1.88)	9.75 (2.46)	12.75 (1.07)
Type 2	3.10 (1.60)	10.92 (1.15)	9.00 (1.04)	13.38 (0.79)
Type 3	1.68 (0.83)	10.65 (1.66)	7.56 (1.95)	13.25 (1.10)

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) <3.0 were to be treated as 1.5.
b) Subjects who received at least one concomitant dose of DPT-sIPV with Hib
c) Subjects who received the fourth dose of DPT-sIPV with Hib

Table 4-16. Adverse events and adverse reactions in subjects who received DPT-sIPV with or without Hib (Study BK-4SP/003, Safety analysis population)

	DPT-sIPV (N = 134)				DPT-sIPV + Hib[a)] (N = 113)			
	Adverse event		Adverse reaction		Adverse event		Adverse reaction	
	n	%	n	%	n	%	n	%
Injection site for DPT-sIPV	98	73.1	98	73.1	92	81.4	92	81.4
Injection sites for DPT-sIPV and Hib	98	73.1	98	73.1	96	85.0	92	81.4
Others	130	97.0	77	57.5	110	97.3	53	46.9
Pyrexia	120	89.6	62	46.3	100	88.5	34	30.1

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction
a) Subjects who received at least one concomitant dose of DPT-sIPV with Hib

PMDA considers as follows:

Although concomitant use with Hib is unlikely to markedly affect the immunogenicity and safety of DPT-sIPV, as there is limited clinical experience of concomitant vaccine administration, it is necessary to actively collect post-marketing safety information on concomitant vaccine administration and investigate its effects as well.

4.B.(7) Post-marketing commitments

The applicant submitted the following post-marketing surveillance plan (draft):

A use-results survey will be conducted. The planned sample size is 750 children who received three doses of DPT-sIPV at 3- to 8-week intervals for primary immunization and 750 children who received a single dose of DPT-sIPV at least 6 months after the primary immunization for booster immunization, at 3 to 90 months of age (3000 doses), which provides a ≥95% probability of detecting at least one case of adverse events with an incidence of 0.1%. The occurrence of adverse reactions, unknown adverse reactions, and factors potentially affecting safety in routine clinical settings can be identified via this survey.

PMDA is currently asking the applicant to explain the basis for the planned sample size and the observation period for vaccine recipients. Based on the applicant's response and the results of the reviews so far, post-marketing commitments etc. will be described in the Review Report (2).

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

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

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.1-2 and 5.3.5.1-3). As a result, noncompliance with the procedures for accountability of the investigational products (the wrong investigational product [the wrong drug number] was dispensed and administered to a subject) and protocol deviations (blood sampling was unnecessarily repeated in the same subject) were found at some trial sites. Although these findings requiring improvement were noted, PMDA concluded that the clinical studies as a whole were conducted in compliance with GCP and there should be no problem with conducting a regulatory review based on the submitted application documents.

IV. Overall Evaluation

As described in “4.B.(2) Efficacy” and “4.B.(3) Safety,” PMDA concluded that the efficacy of DPT-sIPV for the proposed indication has been demonstrated and its safety is acceptable. If it can be concluded at the Expert Discussion that there is no particular problem with the above conclusion, the DPT-sIPV vaccine may be approved.

Review Report (2)

July 12, 2012

I. Product Submitted for Registration

[Brand name]	Tetrabik Subcutaneous Injection Syringe
[Non-proprietary name]	Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine
[Name of applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	December 27, 2011

II. Content of the Review

The Expert Discussion and subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

1. Efficacy and indication

The following conclusion by PMDA was supported by the expert advisors:

Based on the results of evaluation of the immunogenicity of DPT-sIPV against attenuated and virulent strains of poliovirus, the protective antigens of *Bordetella pertussis*, diphtheria toxin, and tetanus toxin in Study BK-4SP/003, the efficacy of DPT-sIPV can be expected and the appropriate indication for the DPT-sIPV vaccine should be "the prevention of pertussis, diphtheria, tetanus, and acute poliomyelitis."

The expert advisor made the following comment:

It must be recognized that there are no direct data showing that DPT-sIPV can prevent the outbreak of polio if wild-type poliovirus enters Japan from polio-prevalent countries, and therefore, polio surveillance in Japan needs to be continued.

2. Safety

Based on all clinical study data submitted, PMDA concluded that the safety profile of DPT-sIPV is tolerable. This decision was supported by the expert advisors.

3. Clinical positioning

PMDA concluded that the DPT-sIPV vaccine can replace the approved Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine (DPT) and Live Oral Poliomyelitis Vaccine (OPV), and this decision was supported by the expert advisors.

At the Expert Discussion, the expert advisor made the following comment:

As mentioned in the Review Report (1), given that the DPT-sIPV vaccine is a vaccine product that will replace the approved OPV, testing the non-inferiority of the immunogenicity of DPT-sIPV to that of OPV in the vaccine development is important [see "4.B.(1) Clinical data package" of Review Report (1)]. Even if simple comparison of neutralizing antibodies in blood between DPT-sIPV and OPV was of little significance from a purely scientific point of view, taking in account of the differences in the immunization schedule and mode of action, given that the DPT-sIPV vaccine was developed as a vaccine product that would replace OPV, it was of clinical significance to obtain the results of direct comparison of DPT-sIPV and OPV. A clinical study should have been designed to explain the clinical positioning of the DPT-sIPV vaccine vs. an existing vaccine, such as comparing the neutralizing antibody titers in blood to the possible extent, though it might have been difficult.

PMDA considers that DPT-sIPV eventually resulted in an adequate neutralizing antibody response in the blood and its clinical positioning is clear, but the expert advisors' comment on novel vaccine development is important. Thus, PMDA explained their concerns to the applicant and the applicant responded that the comment would serve as a reference for future clinical development.

4. Dosage and administration

The following conclusion by PMDA was supported by the expert advisors:

Taking account of the results of Study BK-4SP/003 and the situation in foreign countries where inactivated polio vaccine derived from virulent strains of poliovirus has been introduced [see "4.B.(5).2) Immunization schedule" of Review Report (1)], the dosage and administration statement should be as shown below.

[Dosage and administration]

Primary immunization: The usual primary series for children consist of three doses of 0.5 mL each given by subcutaneous injection at intervals of at least 3 weeks.

Booster immunization:	The usual booster dose for children is a single 0.5 mL dose given by subcutaneous injection at least 6 months after the primary immunization.

At the Expert Discussion, the expert advisors made the following comments:
The normal DPT-sIPV immunization schedule in accordance with the DPT immunization schedule should be described in the Precautions of Dosage and Administration section of the package insert (i.e. the primary series doses should be given at "3- to 8-week intervals" and a booster dose should be given "12 to 18 months after the completion of primary immunization"). It is necessary to take measures to make the appropriate DPT-sIPV immunization schedule informed thoroughly to the healthcare professionals.

PMDA instructed the applicant to modify the dosage and administration statement and the precautions of dosage and administration statement and the applicant responded appropriately. PMDA asked the applicant to consider measures to disseminate the appropriate DPT-sIPV immunization schedule and the applicant responded that they will disseminate the information appropriately, utilizing information leaflets etc.

5. Post-marketing commitments

A use-results survey plan for the DPT-sIPV vaccine was discussed as follows and supported by the expert advisors:
Febrile convulsion is common in the intended population for the DPT-sIPV vaccine. In Study BK-4SP/003, 1 subject (0.4%) had febrile convulsion and 1 subject (0.4%) had convulsion, though not considered as serious adverse events, after vaccination with DPT-sIPV. Hence, it is necessary to design a use-results survey capable of detecting the occurrence of pyrexia, febrile convulsion, and convulsion. For this reason, the planned sample size of a total of 1500 children, 750 children each for primary and booster immunization (which could provide a ≥95% probability of detecting at least one case of adverse events with an incidence of 0.4%) is appropriate. It is also necessary to collect information on the occurrence of clinically significant adverse reactions, e.g. shock and anaphylactoid reaction, which have been noted with the approved DPT and the use of other vaccines coadministered with DPT-sIPV (Freeze-dried Haemophilus Type b Vaccine, Adsorbed Pneumococcal Heptavalent Conjugate Vaccine, etc.).

PMDA instructed the applicant to address the above matter and the applicant responded that they will take appropriate action.

6. Quality

As a result of its review of the application including the applicant's additional explanation, PMDA has concluded that the quality of the candidate vaccine is adequately controlled. In the course of the regulatory review, PMDA instructed the applicant to perform filter integrity test as an in-process control at the sterile filtration step in the detoxification process for the bulk of purified pertussis vaccine [Review Report (1), Table 2-2], the toxoiding process for the bulk diphtheria toxoid [Review Report (1), Table 2-8], the toxoiding process for the bulk tetanus toxoid [Review Report (1), Table 2-14], and the preparation process for trivalent bulk of inactivated poliovirus [Review Report (1), Table 2-19] and the applicant responded appropriately.

6.(1) Raw materials of biological origin

An additional investigation into the country of origin of skim milk and casamino acids used in the preparation of the master seed (MS) for the bulk of purified pertussis vaccine [Review Report (1), Table 2-4] was conducted. As a result, skim milk was found to be derived from cows raised in the US and the country of origin of casamino acids could not be identified, but the supplier's investigation report etc. indicated that casamino acids might have been derived from cows raised in Ireland, Poland, France, Australia, or New Zealand.

The applicant justified the use of casamino acids as follows:

Although the country of origin of casamino acids could not be identified, since it is likely that casamino acids were not sourced from the UK or Portugal, i.e. countries with a high incidence of bovine spongiform encephalopathy (BSE), categorizing the country of origin as "countries where BSE has occurred + countries where there is unknown risk of BSE (countries where BSE has occurred, etc.)," a risk assessment for transmissible spongiform encephalopathy (TSE) was performed in accordance with the attachment to the joint notification of the Evaluation and Licensing Division and the Safety Division, Pharmaceutical and Food Safety Bureau, MHLW (PFSB/ELD-SD Notification No. 0801001, dated August 1, 2003). As a result, the total risk assessment score was −21, which was lower than −3 which is a threshold to provide a certain degree of safety assurance. Therefore, the risk of TSE infection from the candidate DPT-sIPV vaccine is considered very low. Raw materials will be replaced with appropriate ones when a new MS is prepared.

As a result of reviewing the conformance to the guidance document, "Handling of Drugs etc. Produced from Master Cell Banks or Master Seeds That Fail to Meet the Standard for Biological Ingredients" (PFSB/ELD Administrative Notice dated March 27, 2009) in addition to

the results of the risk assessment by the applicant, PMDA concluded that the use of casamino acids is acceptable.The decision was supported by the expert advisors as well.

The applicant also explained that they will immediately consider taking further safety measures (replacement with raw materials subjected to inactivation/removal procedures etc.) for defibrinated bovine blood used in the WS and the seed culture for the bulk of purified pertussis vaccine [Review Report (1), Table 2-4] and equine serum and beef digest used in the WS, seed culture, and the production culture for the bulk diphtheria toxoid [Review Report (1), Table 2-10], since adventitious virus inactivation/removal during the production of these raw materials was unclear.

PMDA accepted the response.

6.(2) Novel excipients

The candidate vaccine contains M199 (Ca, Mg, phosphate, phenol red-free) (M199), which has never been used as an excipient in a medicinal product, and Disodium Edetate Hydrate (JP), which has never been used for subcutaneous injection.

The proposed specifications for M199 include identification, appearance, clarity and color of solution, pH, osmolality, heavy metals, arsenic, bacterial endotoxins, and cytotoxicity. Based on stability studies on monovalent bulks of inactivated poliovirus containing M199, it is stable for [redacted] years at [redacted]°C to [redacted]°C.

Based on the submitted data, PMDA concluded that M199 and Disodium Edetate Hydrate at the levels used in the candidate vaccine are very unlikely to cause a safety problem.

III. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved after modifying the indication and dosage and administration statements as shown below. The re-examination period is 8 years. The drug substance and the drug product are both classified as powerful drugs, and the product is classified as a biological product.

[Indication]

Prevention of pertussis, diphtheria, tetanus, and acute poliomyelitis

[Dosage and administration]

Primary immunization:	The usual primary series for children consist of three doses of 0.5 mL each given by subcutaneous injection at intervals of at least 3 weeks.
Booster immunization:	The usual booster dose for children is a single 0.5 mL dose given by subcutaneous injection at least 6 months after the primary immunization.

(Appendix 1)

The summary of the submitted data and the outline of the review by PMDA regarding master files (MF) for Tetrabik (MF registration numbers, 221MF10287, 221MF10288, 221MF10289, and 222MF10002)

[Brand name]	(a) IPV monovalent bulk of type 1
	(b) IPV monovalent bulk of type 2
	(c) IPV monovalent bulk of type 3
	(d) Trivalent bulk of inactivated polio vaccine
[Non-proprietary name]	(a) Inactivated poliovirus type 1 (Sabin strain)
	(b) Inactivated poliovirus type 2 (Sabin strain)
	(c) Inactivated poliovirus type 3 (Sabin strain)
	(d) Inactivated trivalent polioviruses (Sabin strains)
[Name of submitter]	Japan Poliomyelitis Research Institute
[MF registration numbers]	(a) 221MF10287 (b) 221MF10288 (c) 221MF10289 (d) 222MF10002

A Summary of the submitted data

The drug substance consists of the bulks of inactivated poliovirus types 1, 2, and 3 (monovalent bulks) produced from types 1, 2, and 3 poliovirus (Sabin stains) particles grown in Vero cells, purified, and inactivated with formaldehyde solution. The information contained in each MF is outlined in Figure 1. The details are described in (1) to (6) below.

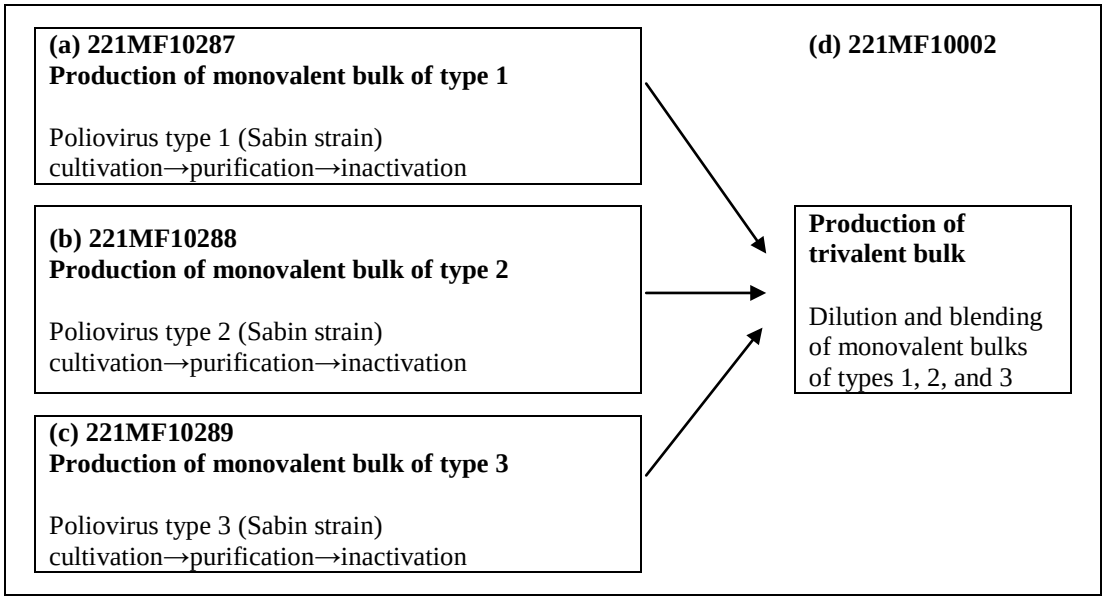


Figure 1. Information contained in MFs

A.(1) Manufacturing process

A.(1).1) Preparation and control of viral seeds

The original seeds derived from poliovirus strains produced by Dr. A. B. Sabin (type 1, strain LS-c, 2ab [redacted]; type 2, strain P712, Ch, 2ab [redacted]; type 3, strain Leon $12a_1b$ [redacted]), distributed by WHO or the National Institute of Health (a predecessor of the National Institute of Infectious Diseases), were passaged [redacted] times in [redacted] [redacted] cells to establish master seeds (MS). The tests to be performed on the MS and the virus passaged [redacted] times from the MS (beyond the passage level used for production) (VAL) are as shown in Table 1.

Table 1. Control tests on viral seeds

Test				MS	WS	VAL
Tests of identity	Virus titration assay (Hep-2c, Kaerber's method)			○	○	○
	Serotyping (antibody neutralization test)			○	—	○
	rct-marker test (temperature sensitivity)			○	—	○
	d-marker test (sodium bicarbonate sensitivity)			○	—	—
	Neurovirulence test			○	—	—
	Gene analysis (nucleotide sequencing)			○	○	○
Tests of purity	Sterility test (thioglycolate medium and SCD medium)			○	○	○
	Mycoplasma testing (direct smear method and enrichment culture method)			○	○	○
	Test for *Mycobacterium tuberculosis*			○	—	○
	Observation of control cells			○	—	—
	Testing of control cells for the presence of hemadsorbing viruses			○	—	—
	Adventitious viruses	*In vitro* assays	Inoculation into green monkey kidney cells	○	○	○
			Inoculation into human diploid cells	○	○	○
			Inoculation into rabbit kidney cells	○	○	○
		In vivo assays	Inoculation into rabbits	○	○	○
			Inoculation into adult mice	○	○	○
			Inoculation into suckling mice	○	○	○
			Inoculation into guinea pigs (intracerebral)	○	○	○
			Inoculation into guinea pigs (intraperitoneal)	○	○	○
	Test for retroviruses (FPERT)			○	—	○

○: Tested, —: Not tested
A single *in vitro* and *in vivo* test for adventitious viruses are performed on the WS.
VAL is tested only when the first MS is prepared.

The MS is stored at ≤[redacted]°C and its expiry period is [redacted] years. The stability during storage will be assessed by performing virus titration assay every [redacted] years. When the number of remaining ampoules of the MS is decreased to a certain level, a new MS will be prepared from the original seed. A newly prepared MS will be qualified by the tests listed in Table 1. Although vaccine production should be based on a two-tiered seed lot system consisting of a MS and a working seed (WS), the MS has been used for the production of the drug substance and a WS has not been generated at present. A two-tiered seed lot system will be introduced in future. A newly prepared WS will be qualified by the tests listed in Table 1.

A.(1).2) Preparation and control of cell banks

Purchased Vero cells (ATCC No.[redacted]; passage number, [redacted]) were propagated to the [redacted]th passage to establish a master cell bank (MCB; passage number, [redacted]) and a working cell bank was prepared from the MCB that had further been grown for [redacted] passages (WCB; passage number,

[redacted]), and the WCB are used for vaccine production. The tests to be performed on the MCB, WCB, and cells cultured beyond the production passage level (CAL; passage number, [redacted]) are as shown in Table 2.

Table 2. Control tests on cell banks

Test					Cell bank to be tested		
					MCB	WCB	CAL
Tests of identity	Cell identity		Isoenzyme analysis		○	○	○
	Cell morphology		Morphological examination by Hematoxylin-Eosin staining		○	—	○
	Cell growth		Cell count measurement		○	—	○
	Tumorigenicity		Observation of tumor formation in mice subcutaneously injected with cell suspension		—	—	○
Tests of purity	Sterility test		Direct inoculation of the culture medium		○	—	—
			Membrane filtration		—	○	○
	Mycoplasma testing		Indicator cell culture method		○	○	○
			Direct inoculation of the culture medium		○	—	—
			Membrane filtration		—	○	○
	Endogenous viruses	Transmission electron microscopy	Observation of viruses or virus-like particles etc.		○	—	○
		Test for retroviruses	Reverse transcriptase activity (FPERT)		○	—	○
		Test for endogenous viruses	NAT by simian immunodeficiency virus-specific PCR		○	—	—
	Adventitious viruses	*In vitro* assays	Cytopathic changes and Hemadsorption/ Hemagglutination	Inoculation into Vero cells (monkey)	○	○	○
				Inoculation into primary kidney cell cultures (monkey)	—	○	—
				Inoculation into RK-13 cells (rabbit)	○	—	○
				Inoculation into primary kidney cell cultures (rabbit)	—	○	—
				Inoculation into MRC-5 cells (human)	○	—	○
				Inoculation into WI-38 cells (human)	—	○	—
		In vivo assays	Inoculation into suckling mice		○	○	○
			Inoculation into adult mice		○	○	○
			Inoculation in embryonated eggs		○	○	○
		Test for human viruses	PCR method (HBV, HCV, HIV)		○	—	○
		Test for bovine viruses	Cytopathic changes and Hemadsorption, Immunofluorescence assay		○	—[a)]	○
		Test for porcine viruses	Cytopathic changes and Immunofluorescence assay		○	—[a)]	○

○: Tested, —: Not tested, CAL is not prepared when a new cell bank is generated.
a) To be performed on a new WCB.

The MCB and WCB have been stored in [redacted] (≤[redacted]°C) and the stability during storage will be assessed by performing viability testing on the WCB every [redacted] years. In addition, cell count will be measured at the thawing of the WCB and in the cell culture process (seed cell culture, expanded cell culture, final cell culture), and virus content will be determined in the virus cultivation process (individual virus suspensions). When the number of remaining ampoules of the MCB or WCB is decreased to a certain level, a new MCB will be prepared from the aforementioned Vero cells (passage number, [redacted]) or Vero cells newly purchased from [redacted], and a new WCB will be prepared from the new MCB. The newly prepared MCB or WCB

will be qualified by the tests listed in Table 2.

A.(1).3) Manufacturing process and critical steps/critical intermediates and process validation

The commercial-scale manufacturing process for monovalent bulk of each serotype is as shown in Table 3.

Table 3. Summary of manufacturing process and controls

Manufacturing process		Intermediate	In-process testing
Cell culture	Seed cell culture 1	MWCB [redacted] mL [redacted] mL, [redacted] °C, [redacted] days	
	Expanded culture 1	[redacted] L, [redacted] °C, [redacted] days	
	Expanded culture 2	[redacted] L, [redacted] °C, [redacted] days	
	Expanded culture 3	[redacted] L, [redacted] °C, [redacted] days	
	Final cell culture	[redacted] L, [redacted] culture [redacted] °C, [redacted] days	Final cell cultures	Control cells (observation of cultures, hemadsorption, inoculation into Vero cells and human cell cultures)
↓			
Virus cultivation		MS inoculation: m.o.i ($CCID_{50}$/cell) Type 1:10[redacted], Type 2: 10[redacted], Type 3: 10[redacted] Cultivation: [redacted] L, [redacted] °C, [redacted] days	Individual virus suspensions	Sterility, Mycoplasma, Virus identity
↓			
Harvest		Filtration (pore size [redacted] µm→[redacted] µm→[redacted] µm)		
		Ultrafiltration (Molecular weight cutoff [redacted])	Concentrate by ultrafiltration	
↓			
Purification		Ultracentrifugation (centrifugation and [redacted] treatment)		
		Anion exchange chromatography	Purified virus solution	
		Dilution Sterile filtration (pore size [redacted] µm)	Filtered virus solution before inactivation	Bovine serum protein content, Host cell protein content, Host cell DNA content, Antibiotic content, Virus content, D-antigen content
↓			
Inactivation		[redacted] w/v % formaldehyde [redacted] °C, [redacted] days [redacted]th day of inactivation: Sterile filtration (pore size [redacted] µm)	Inactivated virus suspension	Absence of residual live virus ([redacted]th day of inactivation)
↓			
Preparation of monovalent bulk		Neutralization of formaldehyde solution /Addition of excipients, pH adjustment	Monovalent bulk solution before filtration	
		Sterile filtration (pore size [redacted] µm)	Filtered monovalent bulk solution	
		Subdividing/Labeling	Drug substance (Monovalent bulk)	

[redacted]: Critical steps or critical intermediates

Monovalent bulks of types 1, 2, and 3 (the drug substance) are diluted, blended, and sterile-filtered ([redacted] µm) to form a trivalent bulk, and the trivalent bulk is distributed into

containers and its storage time is [redacted] months at [redacted]°C.

Process validation was performed on three lots of intermediates or the drug substance manufactured at a pilot-scale and the parameters listed in Table 4 were evaluated. The results have demonstrated that each process step is adequately controlled to ensure consistent production.

Table 4. Process validation/evaluation of manufacturing process for bulk inactivated poliovirus

Process step	Parameter
Cell culture	Final cell cultures (temperature, pH, the amount of blown air, culture duration, cell density [at seeding, at the end of cell culture], control cells [observation, hemadsorption, inoculation into Vero cells])
Virus cultivation	Individual virus suspensions (moi, temperature, air blowing, pH, cultivation duration, cytopathic changes at the end of cultivation, sterility test, mycoplasma testing, virus identity test, test for virus content)
Purification	Ultracentrifugation condition, [redacted] treatment condition, Centrifugation condition, Flow rate, Load volume per gel, Chromatogram, Filtration pressure, D-antigen content after ultracentrifugation, Test for bovine serum protein content, Test for host cell protein content, Test for host cell DNA content, Test for antibiotic content, Test for virus content, Test for D-antigen content, Test for protein content, Protein/D-antigen unit, pH, Filter integrity test, Yield and percent yield of D-antigen in concentrate by ultracentrifugation and in purified virus
Inactivation	Formaldehyde content, Temperature, Reaction time, Protein content before inactivation, test for residual live virus ([redacted]th day and 12th day), Filter integrity test, Reaction time until no virus is detected, Inactivation line
Preparation of monovalent bulk	Cleanliness grade, Filtration temperature, Filtrate volume per unit area of filter, Filtration pressure, Filtration time, Neutralization of formaldehyde solution: pH, Sterile filtration: Sterility test, Test for D-antigen content, Bacterial endotoxins test, Description test, pH, Test for freedom from abnormal toxicity, Test for formaldehyde content, Protein/D-antigen unit, Immunogenicity test, Filter integrity test, and Bacterial challenge testing of filter[a)]
Preparation of trivalent bulk	Sterility test, pH, Test for D-antigen content, Test for freedom from abnormal toxicity, Test for protein content, Test for formaldehyde content, Immunogenicity test, Test for residual live virus, Description test

a) One lot of type 1

A.(1).4) Adventitious agents safety evaluation

The absence of adventitious viruses in all of the MCB, WCB, CAL, MS, and VAL has been confirmed by the tests for viruses listed in Table 1 and Table 2. The raw materials of biological origin used in the manufacturing process are as shown in Table 5.

Table 5. Raw materials of animal origin used in manufacturing process

Process step	Raw material	Animal species	Specific part of animal used	Country of origin
Cell culture	Fetal bovine serum	Bovine	Blood	Australia, New Zealand
	Trypsin	Porcine	Pancreas	
	Lactose (an additive in trypsin)	Bovine	Milk	US
Cell culture Virus cultivation Harvest	Erythromycin lactobionate	Bovine	Milk	US, Netherlands, Belgium, Germany, Luxembourg, or India
Virus cultivation Harvest	Cholesterol (a medium component)	Ovine	Wool	New Zealand, Australia

Fetal bovine serum and porcine pancreas-derived trypsin used in the cell culture process are derived from healthy animals and have been subjected to inactivation treatment (fetal bovine

serum, ≥25 kGy γ-ray irradiation; porcine pancreas-derived trypsin, ≥25 kGy γ-ray irradiation or pH <5.0 for ≥3 hours) and tested for the presence of viruses (fetal bovine serum, bluetongue virus, bovine adenovirus, bovine parvovirus, bovine viral diarrhea virus, bovine RS virus, rabies virus, reovirus, cytopathic agents, and hemadsorbing agents; porcine pancreas-derived trypsin, parvovirus) by the suppliers.

Virus reduction factor of the inactivation step was as shown in Table 6.

Table 6. Virus reduction factor (log_{10}) of inactivation step (12 days)

Virus	Aujeszky's disease virus		Bovine viral diarrhea virus		Canine parvovirus	
	Experiment 1[a)]	Experiment 2[a)]	Experiment 1[a)]	Experiment 2[a)]	Experiment 1[a)]	Experiment 2[a)]
Virus reduction factor (log_{10})	≥ 5.1	≥ 5.1	≥ 5.2	≥ 5.1	≥ 5.0	≥ 5.1

a) Two lots were sampled at the inactivation step ([redacted]th day) and Experiment 1 and Experiment 2 were performed on each lot.

A.(1).5) Manufacturing process development

In [redacted] 20[redacted], a sterile filtration step was introduced into the monovalent bulk preparation process. Bulk solution was tested for protein content and D-antigen content before and after filtration and no differences were observed before and after the manufacturing process change. Based on specification testing results for drug substances, the pre- and post-change products were determined to be comparable, and it was concluded that the change made in the manufacturing process has no impact on quality.

A.(2) Characterization

Characterization was performed by electron microscopy, N-terminal sequencing, cesium chloride density gradient centrifugation, analysis of carbohydrate composition and structure, spectroscopic profiles (ultraviolet and visible absorption spectra), molecular weight and electrophoresis (SDS-PAGE) of structural proteins, gel filtration chromatography, ion-exchange chromatography, immunochemical properties (gel precipitation reaction), and biological properties (immunogenicity studies in rats and [redacted]).

Electron microscopic examination of inactivated virus showed spherical particles with a diameter of about 30 nm and the particles banded at a density of 1.33 to 1.34 g/cm^3 after cesium chloride density gradient centrifugation. The N-terminal amino acid sequence was identical to that in the GenBank database. No glycosylation was detected. The ultraviolet and visible absorption spectra showed a slight peak at about [redacted] nm and bands representing the structural proteins of VP2 (28 kDa) and VP3 (25 kDa) were detected on SDS-PAGE. Not only the main peak apparently representing inactivated poliovirus particles but also peaks due to a neutralizer etc. added in the inactivation step were observed by gel filtration chromatography and

ion-exchange chromatography. In gel precipitation reactions, precipitation line patterns were distinct for each serotype and there was no crossreactivity between serotypes. In immunogenicity studies of monovalent bulks, neutralizing antibody titers rose in a dose-dependent fashion and the neutralizing antibodies elicited were also active against virulent strains.

A.(3) Impurities

The removal of empty particles (poliovirus particles incapable of eliciting neutralizing antibodies) as product-related impurities and of host cell protein, host cell DNA, bovine serum protein, and antibiotics as process-related impurities was investigated. Analysis of an intermediate (Intermediate C) by cesium chloride density gradient centrifugation showed the disappearance of the peak of empty particles. In Intermediate C, host cell protein was reduced to ≤ × 10 ppm, host cell DNA was reduced to < pg/mL, and bovine serum protein was reduced to < ng/mL. The antibiotic level was to (minimum inhibitory dilution ratio) in intermediates before purification (Intermediate A and Intermediate B), but was reduced to < in Intermediate C.

A.(4) Specifications

The drug substance specifications include sterility test, test for D-antigen content, bacterial endotoxins test, description test, test for pH, test for freedom from abnormal toxicity, test for formaldehyde content, test for residual live virus, protein/D-antigen unit, and identity test. The control tests on trivalent bulk include the same tests as the drug substance specification tests excluding the protein/D-antigen unit and bacterial endotoxins test and the immunogenicity test.

A.(5) Standards or reference materials

The reference materials used in the test for D-antigen content are Sabin strains of poliovirus that have been grown in cells for type 1 or in cells for types 2 and 3, purified by , diluted in medium, each distributed into containers. D-antigen contents of the reference materials were determined on the basis of International Reference Standards (obtained from NIBSC [National Institute for Biological Standards and Control]) and the reference materials have been demonstrated to meet the acceptance criteria for the tests for virus content and D-antigen content. The reference materials are stored at ≤°C.

A.(6) Stability

Stability studies on the drug substance are as shown in Table 7.

Table 7. Stability studies on drug substance

Study	No. of lots[a)]	Temperature	Storage period
Long term[b)]	3	[redacted]±[redacted]°C, [redacted]	[redacted] months
Accelerated[c)]	3	[redacted]±[redacted]°C/[redacted]±[redacted]%RH, [redacted]	[redacted] months
Stress (temperature)[d)]	1	[redacted]±[redacted]°C/[redacted]±[redacted]%RH, [redacted]	[redacted] days
Stress (shaking)[e)]	1	[redacted]±[redacted]°C, [redacted], [redacted]rpm	[redacted] hours
Photostability[f)]	1	[redacted]±[redacted]°C, [redacted]lx/hr (1.2 million lx·hr)	[redacted] days

a) Pilot-scale

b) Ultraviolet-visible spectroscopy, gel filtration chromatography, ion-exchange chromatography, cesium chloride density gradient centrifugation, polyacrylamide gel electrophoresis, electron microscopy, test for protein content, and immunogenicity test were performed, in addition to the specification tests excluding identity test.

c) Gel filtration chromatography, ion-exchange chromatography, cesium chloride density gradient centrifugation, electron microscopy, test for protein content, and immunogenicity test were performed, in addition to the specification tests excluding identity test.

d) The tests in the long-term stability study specified in the note b), excluding test for residual live virus and bacterial endotoxins test, were performed.

e) Polyacrylamide gel electrophoresis was performed, in addition to the tests in the accelerated stability study specified in the note c), excluding cesium chloride density gradient centrifugation, electron microscopy, and test for formaldehyde content.

f) The tests in the accelerated stability study specified in the note c), excluding cesium chloride density gradient centrifugation and electron microscopy, were performed.

In the long-term storage condition, although a decrease in potency was observed for all serotypes at [redacted] months, there were no significant changes during storage for all attributes tested and the specifications were met up to [redacted] months. Based on the above, a shelf-life of [redacted] months has been proposed for each monovalent bulk.

B Outline of the review by PMDA

Although PMDA is asking the MF holder to provide a detailed explanation of the manufacturing process and controls for the product and raw materials of biological origin, etc., based on the submitted data, PMDA considers that there are no significant quality problems that would affect the evaluation of non-clinical and clinical studies. The conclusion of the review by PMDA including the MF holder's explanation is outlined in Appendix 2.

(Appendix 2)

The outline of the review regarding master files (MF) for Tetrabik (MF registration numbers, 221MF10287, 221MF10288, 221MF10289, and 222MF10002)

[Brand name]	(a) IPV monovalent bulk of type 1
	(b) IPV monovalent bulk of type 2
	(c) IPV monovalent bulk of type 3
	(d) Trivalent bulk of inactivated polio vaccine
[Non-proprietary name]	(a) Inactivated poliovirus type 1 (Sabin strain)
	(b) Inactivated poliovirus type 2 (Sabin strain)
	(c) Inactivated poliovirus type 3 (Sabin strain)
	(d) Inactivated trivalent polioviruses (Sabin strains)
[Name of submitter]	Japan Poliomyelitis Research Institute
[MF registration numbers]	(a) 221MF10287 (b) 221MF10288 (c) 221MF10289 (d) 222MF10002

B Outline of the review by PMDA

As a result of its review of the applicataion including the MF holder's explanation, PMDA concluded that the quality of the product is adequately controlled.

B.(1) Raw materials of biological origin

The raw materials of biological origin used in the master cell bank (MCB), working cell bank (WCB), and master seed (MS) are as shown in Table 1 and Table 2. As the information on the lots used was destroyed etc. for some of the raw materials, a retrospective investigation was carried out and the information inferred from other lots etc. (shaded entries in Table 1 and Table 2) is presented.

Table 1. Raw materials of animal origin used in the preparation of MCB and WCB

Raw material	Animal species	Specific part of animal used	Country of origin	Inactivation treatment	Test for presence of infectious agents
MCB[a)]					
Bovine serum	Bovine	Blood	US	Sterile filtration	Mycoplasma, Bovine viral diarrhea virus, Infectious bovine rhinotracheitis virus, Parainfluenza virus type 3
Trypsin	Porcine	Pancreas		γ-ray irradiation (≥ 25 kGy)	Bacteria, Fungi, Mycoplasma, Parvovirus
Lactose (an additive in trypsin)	Bovine	Milk	US		
Erythromycin lactobionate	Bovine	Milk	US, Canada, New Zealand		
WCB					
Bovine serum	Bovine	Blood	New Zealand	Sterile filtration	Bacteria, Fungi, Mycoplasma, Hemadsorbing agents, Cytopathic agents
Trypsin	Porcine	Pancreas		None	None
Erythromycin lactobionate	Bovine	Milk	US, Canada, New Zealand		

a) MCB was prepared in 19[redacted] (before BSE was first reported in the US in 2003).

Table 2. Raw materials of animal origin used in MS preparation

Raw material		Animal species	Specific part of animal used	Country of origin	Inactivation treatment	Test for presence of infectious agents
MS[a)]						
Bovine serum		Bovine	Blood	US	Sterile filtration	Mycoplasma, Bovine viral diarrhea virus, Infectious bovine rhinotracheitis virus, Parainfluenza virus type 3
Trypsin		Porcine	Pancreas		γ-ray irradiation (≥ 25 kGy)	Bacteria, Fungi, Mycoplasma, Parvovirus
Lactose (an additive in trypsin)		Bovine	Milk	US		
Erythromycin lactobionate	Type 1[b)] Type 2[b)]	Bovine	Milk	US, Canada, New Zealand		
	Type 3[b)]	Bovine	Milk	US, Canada, New Zealand		
Lactose (an additive in Dispase)	Type 1[b)] Type 2[b)]	Bovine	Milk	Netherlands, Belgium, Germany, Luxembourg		
Lactalbumin hydrolysate	Type 1[b)] Type 2[b)]	Bovine	Milk	Australia, New Zealand	215°F for 30 seconds, 160°F for 3 hours	None
	Type 3[b)]	Bovine	Milk	US	140°C for ≥ 8 hours, 110°C for ≥ 15 seconds	Unknown
Pancreas-derived enzyme (lactalbumin hydrolysate)	Type 1[b)] Type 2[b)]	Porcine	Pancreas		215°F for 30 seconds, 160°F for 3 hours	None
	Type 3[b)]	Porcine	Pancreas		High temperature	Unknown
Gelatin	Type 3[b)]	Porcine	Bone			

a) MS of polioviruses of serotypes 1 and 2 were prepared in 19[redacted] (before BSE was first reported in the US in 2003) and MS of poliovirus of serotype 3 was prepared in 19[redacted] (before BSE was first reported in the UK in 1986).
b) Raw materials used in MS preparation are listed by serotype if different ones were used.

As adventitious viral safety has been assured by the ability of the detoxification, inactivation, or purification step etc. of the manufacturing process for the drug substance to remove viruses and

the information inferred from the retrospective investigation indicates the conformance to the Standard for Biological Ingredients, PMDA concluded that these raw materials may be used. The above conclusion by PMDA was supported by the expert advisors.

The MF holder responded that they will immediately consider further safety measures (replacing with raw materials subjected to inactivation/removal procedures etc.) to be taken for bovine serum and trypsin used in the WCB preparation since adventitious virus inactivation/removal during the production of these raw materials was unclear, and PMDA accepted the response. Since the ability of the treatment to inactivate viruses etc. was unclear, trypsin treated with "pH <5.0 for ≥3 hours" used in the cell culture process, listed in Table 5 of Appendix 1, has been replaced with trypsin treated with "≥25 kGy γ-ray irradiation."

B.(2) Reference materials

For renewal of the reference materials used in the test for D-antigen content, a D-antigen content of a new reference material is determined on the basis of the current reference material.

PMDA instructed the MF holder to calibrate a new working reference material against a primary reference material whose D-antigen content has been uniquely assigned, in order to further increase the accuracy of the measurement of D-antigen content. The MF holder responded that they will address it.