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]	Quattrovac Subcutaneous Injection Syringe
[Non-proprietary name]	Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine
[Applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	January 27, 2012

[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.

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]	Quattrovac Subcutaneous Injection Syringe
[Non-proprietary name]	Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine
[Name of applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	January 27, 2012
[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, ≤16.7 Lf of diphtheria toxoid, ≤6.7 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. 0127-15 of Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated January 27, 2012)
[Reviewing office]	Office of Biologics II

Review Results

July 12, 2012

[Brand name] Quattrovac Subcutaneous Injection Syringe

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

[Name of applicant] The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)

[Date of application] January 27, 2012

[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 13, 2012

I. Product Submitted for Registration

[Brand name] Quattrovac Subcutaneous Injection Syringe

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

[Name of applicant] The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)

[Date of application] January 27, 2012

[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, ≤16.7 Lf of diphtheria toxoid, ≤6.7 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. 0127-15 of Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated January 27, 2012)

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 DPT "KAKETSUKEN" Syringe (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 2002, 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, ≤16.7 Lf of diphtheria toxoid, and ≤6.7 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 an adjuvant composed of aluminum chloride and sodium hydroxide.

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 Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW (PFSB/ELD Notification No. 0127-15 dated January 27, 2012, "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 hydroxide as an adjuvant.

2.A.(1) Drug substance

The drug substance consists of the bulk of purified pertussis vaccine, bulk purified diphtheria toxoid, and bulk purified 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 purified diphtheria toxoid, and bulk purified 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 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 cultured to prepare a pre-master seed of *Bordetella pertussis*. The pre-master seed was passaged [redacted] times and frozen to establish a master seed (MS) in 20[redacted]. The MS was passaged [redacted] times and frozen to prepare a working seed (WS). The MS, WS, and the bacterium passaged beyond the level used in production (ES) conformed to the tests listed in Table 2-1 and the seeds were qualified.

Table 2-1. Tests on *Bordetella pertussis* seed banks

Test	MS	WS	ES[a)]
Staining test (Gram's method)	○	○	－
Purity test (culture method and Gram staining)	○	○	－
Nucleotide sequence analysis	○	－	○
Bacterial growth analysis	○	○	○
Antigen production assay (PT)	○	○	○
Antigen production assay (FHA)	○	○	○

○: Tested, －: Not tested

a) Bacterial suspension prepared by [redacted] passages from the WS.

The MS and WS have been stored at ≤[redacted]°C and the stability of the MS and WS during storage will be assessed by performing bacterial growth analysis, antigen production assay (PT), and antigen production assay (FHA) every [redacted] years, or at the time of use. When the number of remaining vials of the MS or WS is decreased to a certain level, a new MS will be prepared from the pre-master seed of *Bordetella pertussis* 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 and controls for the bulk of purified pertussis vaccine are summarized 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
Seed culture 1	WS [redacted] mL [redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture		Seed cultures 1	
Seed culture 2	[redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture		Seed cultures 2	
Main culture	[redacted] – [redacted] L, [redacted] ± [redacted] °C, [redacted] – [redacted] hours, [redacted] culture		Main cultures	Staining, PT antigen content, FHA antigen content
↓				
[redacted]	Add [redacted] to culture supernatants Filtration (pore size [redacted] μm)			
	[redacted] chromatography	Eluted fraction	First eluate of PT (→**(a)**)	
		Flow-through fraction	First FHA solution to be applied (→**(b)**)	
	(a) Dialysis of **First eluate of PT**		First purified PT solution	
[redacted]	Dilution, [redacted] treatment		Second PT solution to be applied	
	[redacted] chromatography		Second eluate of PT	
	Dialysis		Second purified PT solution	Purity (specific activity), Endotoxins
↓				
PT detoxification	Dilution, pH adjustment		PT solution before detoxification	
	Sterile filtration (pore size [redacted] μm)			Filter integrity
	[redacted] – [redacted] vol % formalin treatment [redacted] °C, [redacted] days			
↓				
Preparation of purified PT bulk	[redacted] treatment			
	Dialysis		Purified PT bulk (→**(c)**) Storage condition [redacted] ± [redacted] °C, [redacted] months	Sterility, Mouse histamine sensitization, Mouse body weight decreasing toxicity, Protein nitrogen content, Formaldehyde content, Absence of residual activity of heat-labile toxin
[redacted]	**(b)** Apply **First FHA solution** to [redacted] chromatography		First purified FHA solution	
[redacted]	Dilution, [redacted] treatment		Second FHA solution to be applied	
	[redacted] chromatography		Second purified FHA solution	Purity (specific activity), Endotoxins, PT antigen content
↓				
FHA detoxification	Dilution		FHA solution before detoxification	
	Sterile filtration (pore size [redacted] μm)			Filter integrity
	[redacted] – [redacted] vol % formalin treatment [redacted] °C, [redacted] days			
↓				
Preparation of purified FHA bulk	[redacted] treatment			
	Dialysis		Purified FHA bulk (→**(c)**) Storage condition, [redacted] ± [redacted] °C, [redacted] months	Sterility, Mouse histamine sensitization, Mouse body weight decreasing toxicity, Protein nitrogen content, Formaldehyde content, Absence of residual activity of heat-labile toxin
Preparation of bulk of purified pertussis vaccine	Mixing of **(c) Purified PT bulk** and **(c) Purified FHA bulk**		Pertussis bulk (bulk of purified pertussis vaccine)	

[redacted]: Critical steps or critical intermediates

Process validation (control of operational parameters of critical steps and quality control tests of critical intermediates) was performed on three commercial-scale lots each of the purified PT bulk and the purified FHA bulk and three pilot-scale lots of bulk of purified pertussis vaccine, which demonstrated the robustness of the manufacturing process and the consistency of the quality of critical intermediates. In the PT and FHA detoxification steps, PT and FHA were demonstrated to be appropriately detoxified even under worst-case conditions. As both PT and FHA are [redacted] by formalin treatment, sterile filtration can not be performed in the subsequent steps. Thus, the media fill test was conducted to validate aseptic processing.

(c) Adventitious agents safety evaluation

The absence of adventitious agents in the MS and WS has been confirmed by the staining test and purity test listed in Table 2-1.

The raw materials of biological origin used in the production of the bulk of purified pertussis vaccine are as shown in Table 2-3.

Table 2-3. Raw materials of biological 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 preparation	Peptone	Porcine	Stomach	
MS preparation	Pancreatin	Porcine	Pancreas	
MS preparation	Bovine blood	Bovine	Blood	Australia, New Zealand
MS preparation, WS preparation, Seed culture 1, Seed culture 2, Main culture	Casamino acids	Bovine	Milk	US, Australia, New Zealand
[redacted] chromatography [redacted]	Apoceruloplasmin	Human	Blood	

Peptone, pancreatin, and casamino acids have been autoclaved during the preparation of media containing these raw materials. The plasma that served as the source of apoceruloplasmin has been tested negative for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by PCR. In addition, virus inactivation/removal procedures for apoceruloplasmin include cold-ethanol fractionation (-[redacted]°C, pH[redacted], [redacted]% ethanol), heat treatment ([redacted]°C, [redacted] hours), and virus removal membrane filtration (pore size [redacted] nm). The results of evaluation of cold-ethanol fractionation (-[redacted]°C, pH[redacted], [redacted]% ethanol) for viral clearance are shown in Table 2-4.

Table 2-4. Results of evaluation of apoceruloplasmin manufacturing process for viral clearance

Process step	Virus reduction factor (log_{10})			
	Pseudorabies virus	Porcine parvovirus	Bovine viral diarrhea virus	Murine encephalomyocarditis virus
Cold-ethanol fractionation	5.0	1.9	4.7	2.3

The results of evaluation of the pertussis bulk manufacturing process for viral clearance were as shown in Table 2-5.

Table 2-5. Results of evaluation of manufacturing process for bulk of purified pertussis vaccine for viral clearance[a)]

Process step	Virus reduction factor (log_{10})					
	Herpes simplex virus type 1		Japanese encephalitis virus		Poliovirus type 1 (Sabin strain)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
PT detoxification	> 4.22	> 4.44	> 4.20	> 4.27	> 5.39	> 5.36
FHA detoxification	> 3.90	> 3.90	> 4.22	> 4.02	> 5.29	> 5.02

a) Taking account of the cytotoxicity of PT and FHA solutions before detoxification, viral clearance studies were performed using detoxified PT and FHA solutions.

2.A.(2).2) Characterization

The nucleotide sequences of the PT and FHA genes were identical to those in the database and the amino acid compositions and N-terminal amino acid sequences of purified PT and FHA also agreed with the values and sequences deduced from the nucleotide sequences. Carbohydrate analysis of PT and FHA showed that they are not glycosylated.

The bands corresponding to the S1 to S5 subunits of PT (26 kDa, 22 kDa, 21 kDa, 13 kDa, 14 kDa) were detected on SDS-PAGE of the second purified PT solution. A single 220 kDa band corresponding to FHA was detected on SDS-PAGE of the second purified FHA solution. Analysis of the purified PT bulk and the purified FHA bulk after formalin treatment both yielded a high molecular weight band apparently representing [redacted]. [redacted] rates for three lots of the purified PT bulk and for three lots of the purified FHA bulk were [redacted]% to [redacted]% and [redacted]% to [redacted]%, respectively. No lot-to-lot differences in [redacted] of [redacted] were observed for 8 lots of the purified PT bulk and 9 lots of the purified FHA bulk. The lots for each bulk consisted of the three lots mentioned above, the lots used in the production of investigational products, and the lots of the bulk detoxified under worst-case conditions.

The particle sizes of the purified PT bulk, the purified FHA bulk, and the bulk of purified pertussis vaccine were [redacted] to [redacted]μm (modal particle size, [redacted]-[redacted]μm), [redacted] to [redacted]μm (modal particle size, [redacted]-[redacted]μm), and [redacted] to [redacted]μm (modal particle size, [redacted]-[redacted]μm), respectively. There were no lot-to-lot differences in the particle size distribution pattern of each

intermediate or the pertussis bulk, and an absorption peak at about [redacted]nm with a shoulder at about [redacted]nm was observed.

Protection against symptoms induced by *Bordetella pertussis* infection was demonstrated by mice immunized with the bulk of purified pertussis vaccine [see "3.(i).A.(1) Primary pharmacodynamics"].

2.A.(2).3) Impurities

As process-related impurities, *Bordetella pertussis* protein impurities, *Bordetella pertussis* DNA, and formaldehyde were demonstrated to be removed by ≥[redacted]%. The apoceruloplasmin content and the endotoxin content after the purification process were ≤[redacted] ng per dose and ≤[redacted] EU per dose, respectively.

2.A.(2).4) Specifications

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

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

As the standards, the Reference 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 and mouse body weight decreasing toxicity test, respectively. Each standard is stored at [redacted]±[redacted]°C.

As in-house reference materials, portions of the second purified PT solution and the second purified FHA solution produced at a commercial scale are used in the test for PT antigen content and the test for FHA antigen content, respectively. Each in-house reference material is characterized by protein nitrogen content, calibration curve analysis for antigen content determination, SDS-PAGE electrophoretic pattern, and absorption spectrum and stored at ≤[redacted]°C. For renewal of in-house reference materials, the following points will be checked: the slopes of the calibration curves for the old and new reference materials (mean ± [redacted]SD) are the same; and when the old and new reference materials are used for determining the antigen content of the same sample, the measurements (mean ± [redacted]SD) agree with each other.

2.A.(2).6) Stability of pertussis bulk

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

Table 2-6. Stability studies on bulk of purified pertussis vaccine

Study		No. of lots	Storage condition	Storage package	Storage period
Long-term		3	± °C, protected from light	Stainless container	months[a)]
Accelerated		3	25 ± 2°C, ± %RH, protected from light		months
Stress	Temperature	1	°C, °C, °C, protected from light		days
	Shaking	1	± °C, rpm, protected from light		hours

a) The study is ongoing.

At the long-term storage condition, there were no changes over time up to months. At the accelerated and stress conditions, decreases in protein nitrogen content, the occurrence of insoluble material and associated clarification, decreased high molecular weight bands and increased low molecular weight bands on SDS-PAGE, and increases in particle size were observed and the pertussis bulk was unstable to high temperature and shaking.

Based on the above, a shelf-life of months has been proposed for the pertussis bulk when it is stored in stainless containers at ± °C. The long-term stability study will be continued up to months.

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

The diphtheria bulk is a purified 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 cultured to prepare a pre-master seed of *Corynebacterium diphtheriae* and the pre-master seed was passaged to times and frozen in 20 and 20 to establish a MS. The MS was passaged times and frozen to prepare a WS. The MS, WS, and ES conformed to the tests listed in Table 2-7 and the seeds were qualified.

Table 2-7. Tests on *Corynebacterium diphtheriae* seed banks

Test	MS	WS	ES[a)]
Staining test (Gram's method)	○	○	－
Purity test (culture method and Gram staining)	○	○	－
Nucleotide sequence analysis	○	－	○
Bacterial growth analysis	○	○	○
Antigen production assay	○	○	○

○:Tested, －: Not tested

a): Bacterial suspension prepared by passages from the WS

The MS and WS have been stored at ≤ °C. The stability of the MS and WS during storage will be assessed by performing bacterial growth analysis and antigen production assay every years, or at the time of use. When the number of remaining vials of the MS or WS is decreased

to a certain level, a new MS will be prepared from the pre-master seed of *Corynebacterium diphtheriae* 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 and controls for the diphtheria bulk are summarized in Table 2-8.

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

Manufacturing process		Intermediate/Final	In-process testing
Seed culture 1	WS (inoculated at OD_{650} = [redacted]) [redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture	Seed cultures 1	
Seed culture 2	[redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture	Seed cultures 2	
Main culture	[redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture	Main cultures	Staining
	Centrifugation	Supernatant after centrifugation	
	Filtration (pore size [redacted] µm)	Toxin solution	Antigen content
↓			
Ammonium sulfate precipitation	[redacted] filtration (Molecular weight cutoff [redacted])	Concentrate	
	Ammonium sulfate precipitation I ([redacted]% saturation)	Fraction I solution	
	Ammonium sulfate precipitation II ([redacted]% saturation)	Crude purified solution	
[redacted]	[redacted] chromatography	First purified solution	
[redacted]	[redacted] chromatography	Second eluate	
	Dialysis	Purified toxin solution	Purity
↓			
Toxoiding	Dilution	Solution before toxoiding	
	Filtration (pore size [redacted] µm)		
	[redacted] - [redacted] vol % formalin treatment, [redacted] °C, [redacted] days	Toxoid solution	
↓			
Preparation of bulk purified diphtheria toxoid	Dialysis		
	Sterile filtration (pore size [redacted] µm)	Diphtheria bulk (bulk purified diphtheria toxoid)	Filter integrity

[redacted]: Critical steps or critical intermediates

Process validation (control of operational parameters of critical steps and quality control tests of critical intermediates) was performed on three commercial scale lots of the bulk purified diphtheria toxoid. The test results demonstrated the robustness of the manufacturing process and the consistency of the quality of critical intermediates. As reversion to toxicity occurred when the toxin was formalin-treated for [redacted] days in the toxoiding step, "[redacted] to [redacted] days" of formalin treatment has been selected for irreversible toxoiding.

(c) Adventitious agents safety evaluation

The absence of adventitious agents in the MS and WS has been confirmed by the staining test and purity test listed in Table 2-7.

The raw materials of biological origin used in the production of the bulk purified diphtheria toxoid are as shown in Table 2-9.

Table 2-9. Raw materials of biological origin used in the production of bulk purified diphtheria toxoid

Process step	Raw material	Animal species	Specific part of animal used	Country of origin
MS preparation	Peptone	Porcine	Stomach	
MS preparation	Beef	Bovine	Meat	Australia, New Zealand
MS preparation	Equine serum	Equine	Blood	
MS preparation, WS preparation, Seed culture 1, Seed culture 2, Main culture	Casamino acids	Bovine	Milk	US, Australia, New Zealand

Peptone, beef, and casamino acids have been autoclaved during the preparation of media containing these raw materials. Equine serum has been tested for the presence of viral contaminants (bovine viral diarrhea virus, rabies virus, reovirus, equine herpes virus, equine viral arteritis virus) by fluorescent antibody technique, and has been tested for cytopathic and hemadsorbing viruses as well.

The results of evaluation of the diphtheria bulk manufacturing process for viral clearance were as shown in Table 2-10.

Table 2-10. Results of evaluation of manufacturing process for bulk purified diphtheria toxoid for viral clearance[a)]

Process step	Virus reduction factor (log_{10})					
	Herpes simplex virus type 1		Japanese encephalitis virus		Poliovirus type 1 (Sabin strain)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Toxoiding step	> 4.45	> 4.53	> 4.38	> 4.55	> 4.99	> 5.09

a) Taking account of the cytotoxicity of solution before toxoiding, viral clearance studies were performed using toxoid solution.

(d) Manufacturing process development

A [redacted]-tiered seed lot system consisting of MS and WS was introduced in order to ensure consistent production of the diphtheria bulk. One lot of diphtheria bulk and intermediates produced using a [redacted]-tiered seed lot system was demonstrated to conform to all in-process tests and specification tests except for the test for antigen content of the diphtheria bulk. The test results were within the variation range of three lots of pre-change product, except for the purity, antigen content, and formaldehyde content of the diphtheria bulk. The antigen content of the post-change diphtheria bulk ([redacted] Lf/mL) fell below the lower specification limit ([redacted] Lf/mL),

which was considered attributable to a [redacted]-times lower concentration than usual in the bulk purified diphtheria toxoid preparation step.

2.A.(3).2) Characterization

The nucleotide sequence of the diphtheria toxin gene was identical to that in the database, and the amino acid composition and N-terminal amino acid sequence of the purified toxin solution also agreed with the values and sequence deduced from the nucleotide sequence. Carbohydrate analysis of the diphtheria toxin showed that it is not glycosylated.

A 54 kDa band corresponding to diphtheria toxin was detected on SDS-PAGE of the purified toxin solution and a 45- to 90-kDa broad band was detected on SDS-PAGE of the bulk purified diphtheria toxoid after formalin treatment. The purified toxin solution had an isoelectric point of about [redacted] and the bulk purified diphtheria toxoid had an isoelectric point of about [redacted] to [redacted], as determined by two-dimensional electrophoresis. The purified toxin solution had an absorption peak at about [redacted]nm and the bulk purified diphtheria toxoid had absorption peaks at about [redacted]nm and about [redacted]nm. Analysis of the purified toxin solution or the bulk purified diphtheria toxoid by gel filtration chromatography yielded [redacted] peaks and analysis of the purified toxin solution or the bulk purified diphtheria toxoid by ion exchange chromatography yielded [redacted] peaks ([redacted] was detected in the purified toxin solution).

There were no lot-to-lot differences in the antigenicity of the purified toxin solution (four lots) or the bulk purified diphtheria toxoid (four lots) as measured by double immunodiffusion and their purities (antigen content per mg of protein nitrogen) were [redacted] to [redacted] Lf/mg and [redacted] to [redacted] Lf/mg, respectively. The minimum concentration of the purified toxin solution required to produce a cytopathic effect on Vero cells (exposure period, [redacted] days) was [redacted] pg/mL while the bulk purified diphtheria toxoid did not kill the cells even at [redacted]pg/mL.

When mice were immunized with the bulk purified diphtheria toxoid or the formulated bulk (containing aluminum gel), aluminum gel was shown to increase the level of neutralizing antibody elicited by diphtheria toxoid [redacted]-fold.

2.A.(3).3) Impurities

As process-related impurities, *Corynebacterium diphtheriae* protein impurities and *Corynebacterium diphtheriae* DNA were demonstrated to be removed by ≥[redacted]% and formaldehyde was demonstrated to be removed by ≥[redacted]%.

2.A.(3).4) Specifications

The specifications for the diphtheria bulk include purity test, sterility test, detoxification test, bacterial endotoxins test, test for formaldehyde content, test for antigen content, and potency test.

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

As the standards, the Standard Diphtheria Toxoid and the Standard Diphtheria Antitoxin distributed by NIID are used in the potency test and stored at [redacted]±[redacted]°C. As test toxins, the Diphtheria Test Toxin (for Cell Culture) and the Diphtheria Test Toxin (for rabbit) distributed by NIID are used in the potency test and the Schick Test Toxin (for animal use) distributed by NIID is used in the detoxification test (in rabbits). The test toxins are stored at [redacted]±[redacted]°C.

As an in-house reference material, appropriate protease-treated immunoglobulin fraction of plasma or serum from immunized horse is used in the test for antigen content and stored at [redacted]±[redacted]°C. This in-house reference material is calibrated in diphtheria antitoxin units against the Reference Diphtheria Antitoxin (for flocculation test) distributed by NIID every [redacted] years.

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

Stability studies on the diphtheria bulk are as shown in Table 2-11.

Table 2-11. Stability studies on bulk purified diphtheria toxoid

Study		No. of lots	Storage condition	Storage package	Storage period
Long term		3	[redacted]±[redacted]°C, protected from light	Stainless container	[redacted] months[a)]
Accelerated		3	25±2°C, [redacted]±[redacted]%RH, protected from light		[redacted] months
Stress	Temperature	1	[redacted]°C, [redacted]°C, [redacted]°C, protected from light		[redacted] days
	Shaking	1	[redacted]±[redacted]°C, protected from light		[redacted] hours

a) The study is ongoing.

At the long-term storage condition, there were no changes over time up to [redacted] months. At the accelerated and stress (temperature) conditions, gel filtration chromatography showed that the peak of dimeric diphtheria toxoid increased. Significant changes in potency, antigen content, gel precipitation reaction, etc. were observed especially at the storage temperature of [redacted]°C.

Based on the above, a shelf-life of [redacted] months has been proposed for the diphtheria bulk when it is stored in stainless containers at [redacted]±[redacted]°C. The long-term stability study will be continued up to [redacted] months.

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

The tetanus bulk is a purified 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 A/47 strain of *Clostridium tetani* distributed by NIID was cultured to prepare a pre-master seed of *Clostridium tetani* and the pre-master seed was passaged [redacted] times and frozen to establish a MS in 20[redacted]. The MS was passaged [redacted] times and frozen to prepare a WS. The MS, WS, and ES conformed to the tests listed in Table 2-12 and the seeds were qualified.

Table 2-12. Tests on *Clostridium tetani* seed banks

Test	MS	WS	ES[a)]
Staining test (Gram's method)	○	○	－
Purity test (culture method and Gram staining)	○	○	－
Nucleotide sequence analysis	○	－	○
Bacterial growth analysis	○	○	○
Antigen production assay	○	○	○

○: Tested, －: Not tested

a) Bacterial suspension prepared by [redacted] passages from the WS

The MS and WS have been stored at ≤[redacted]°C and the stability of the MS and WS during storage will be assessed by performing bacterial growth analysis and antigen production assay every [redacted] years, or at the time of use. When the number of remaining vials of the MS or WS is decreased to a certain level, a new MS will be prepared from the pre-master seed of *Clostridium tetani* 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 2-12.

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

The manufacturing process and controls for the bulk purified tetanus toxoid are summarized in Table 2-13.

Table 2-13. Summary of manufacturing process and controls for bulk purified tetanus toxoid

Manufacturing process		Intermediate/Final	In-process testing
Seed culture	WS [redacted] mL [redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture	Seed cultures	
Main culture	[redacted] L, [redacted] ± [redacted] °C, [redacted] hours, [redacted] culture	Main cultures	Staining
	Filtration (pore size [redacted] μm→[redacted] μm)	Toxin solution	Antigen content
↓			
Ammonium sulfate precipitation	[redacted] filtration (Molecular weight cutoff [redacted])	Concentrate	
	Ammonium sulfate precipitation I ([redacted]% saturation)	Fraction I solution	
	Ammonium sulfate precipitation II ([redacted]% saturation)	Crude purified solution	
[redacted]	[redacted] chromatography	First purified solution	
[redacted]	[redacted] chromatography	Second eluate	
[redacted]	Dialysis	Purified toxin solution	Purity
↓			
Toxoiding	Dilution	Solution before toxoiding	
	Filtration (pore size [redacted] μm)		
	[redacted] - [redacted] vol % formalin treatment ([redacted] °C, [redacted] days)	Toxoid solution	
↓			
Preparation of bulk purified tetanus toxoid	Dialysis		
	Sterile filtration (pore size [redacted] μm)	Tetanus bulk (bulk purified tetanus toxoid)	Filter integrity

[redacted]: Critical steps or critical intermediates

Process validation (control of operational parameters of critical steps and quality control tests of critical intermediates) was performed on three commercial scale lots of the bulk purified tetanus toxoid, which demonstrated the robustness of the manufacturing process and the consistency of the quality of critical intermediates. In the toxoiding step, the toxin was demonstrated to be toxoided appropriately even under worst-case conditions.

(c) Adventitious agents safety evaluation

The absence of adventitious agents in the MS and WS has been confirmed by the staining test and purity test listed in Table 2-12.

The raw materials of biological origin used in the production of the bulk purified tetanus toxoid are as shown in Table 2-14.

Table 2-14. Raw materials of biological origin used in the production of bulk purified tetanus toxoid

Process step	Raw material	Animal species	Specific part of animal used	Country of origin
MS preparation	Beef	Bovine	Meat	Australia, New Zealand
MS preparation	Bovine liver	Bovine	Liver	Australia, New Zealand
MS preparation	Polypepton	Bovine	Milk	US, Australia, New Zealand, Poland, China
MS preparation	Pancreatin	Porcine	Pancreas	
MS preparation, WS preparation, seed culture, main culture	Peptone	Porcine	Stomach	
MS preparation, WS preparation, seed culture, main culture	Heart extract	Whale	Heart	

As the viral safety of all of the raw materials of biological origin is assured by autoclaving during the preparation of media, no viral clearance studies for the tetanus bulk manufacturing process have been performed.

2.A.(4).2) Characterization

The nucleotide sequence of the tetanus toxin gene was identical to that in the database and the amino acid composition and N-terminal amino acid sequence of the purified toxin solution also agreed with the values and sequence deduced from the nucleotide sequence. Carbohydrate analysis of the tetanus toxin showed that it is not glycosylated.

An 85 kDa band corresponding to the H-chain of tetanus toxoid and a 47 kDa band corresponding to the L-chain of tetanus toxoid were detected on SDS-PAGE of the purified toxin solution. A 150 kDa broad band was detected on SDS-PAGE of the bulk purified tetanus toxoid after formalin treatment. The purified toxin solution had isoelectric points of about [redacted] to [redacted] (presumably H-chain) and about [redacted] (presumably L-chain). The bulk purified tetanus toxoid had an isoelectric point of about [redacted] to [redacted], as determined by two-dimensional electrophoresis. The purified toxin solution and the bulk purified tetanus toxoid had an absorption peak at about [redacted]nm. Analysis of the purified toxin solution and the bulk purified tetanus toxoid by gel filtration chromatography yielded [redacted] peaks of tetanus toxin and [redacted] peaks of tetanus toxoid, respectively. Analysis of the purified toxin solution and the bulk purified tetanus toxoid by ion exchange chromatography yielded [redacted] peaks of tetanus toxin and [redacted] peaks of tetanus toxoid, respectively.

There were no lot-to-lot differences in the antigenicity of the purified toxin solution (four lots) or the bulk purified tetanus toxoid (four lots) as measured by double immunodiffusion. The purities of the purified toxin solution (five lots) and the bulk purified tetanus toxoid (five lots) (antigen content per mg of protein nitrogen) were [redacted] to [redacted]Lf/mg and [redacted] to [redacted]Lf/mg, respectively.

Guinea pigs were injected subcutaneously with the purified toxin solution or the bulk purified tetanus toxoid in the thigh (n = 2 per group). As a result, all guinea pigs injected with ≥[redacted]ng/dose of the purified toxin solution died within [redacted] days and limb rigidity was observed at [redacted]ng/dose. On the other hand, no abnormalities were found in guinea pigs injected with the bulk purified tetanus toxoid up to [redacted]mg/dose during the [redacted]-day observation period. When mice were immunized with the bulk purified tetanus toxoid or the formulated bulk (containing aluminum gel), aluminum gel was shown to increase the protection against tetanus toxin by [redacted]-fold.

2.A.(4).3) Impurities

As process-related impurities, *Clostridium tetani* protein impurities and *Clostridium tetani* DNA were demonstrated to be removed by ≥[redacted]% and formaldehyde was demonstrated to be removed by ≥[redacted]%.

2.A.(4).4) Control of tetanus bulk

The specifications for the bulk purified tetanus toxoid include purity test, sterility test, detoxification test, bacterial endotoxins test, test for formaldehyde content, test for antigen content, and potency test.

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

As the standard and a test toxin, the Standard Tetanus Toxoid and the Tetanus Test Toxin distributed by NIID are used in the potency test and stored at [redacted]± [redacted]°C.

As an in-house reference material, appropriate protease-treated immunoglobulin fraction of plasma or serum from immunized horses is used in the test for antigen content and stored at [redacted]± [redacted]°C. This in-house reference material is calibrated in tetanus antitoxin units against the Reference Tetanus Antitoxin (for flocculation test) distributed by NIID every [redacted] years.

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

Stability studies on the tetanus bulk are as shown in Table 2-15.

Table 2-15. Stability studies on bulk purified tetanus toxoid

Study		No. of lots	Storage condition	Storage package	Storage period
Long term		3	[redacted]± [redacted]°C, protected from light	Stainless container	[redacted] months[a)]
Accelerated		3	25 ± 2°C, [redacted]± [redacted]%RH, protected from light		[redacted] months
Stress	Temperature	1	[redacted]°C, [redacted]°C, [redacted]°C, protected from light		[redacted] days
	Shaking	1	[redacted]± [redacted]°C, protected from light		[redacted] hours

a) The study is ongoing.

At the long-term storage condition, there were no changes over time up to [redacted] months of storage. At the accelerated and stress (temperature) conditions, a band shift to a higher molecular weight and/or a lower molecular weight on SDS-PAGE and an increase in the dimer peak on gel filtration chromatography were observed. At the stress condition (stored at [redacted]°C), there were significant changes in potency, absorption spectrum, tetanus toxoid content, etc. At the stress condition (shaking), [redacted] guinea pigs died, but showed no symptoms specific to tetanus toxin in the detoxification test using the sample after shaking (without incubation at an elevated temperature). Thus, the deaths were considered unrelated to shaking of the sample.

Based on the above, a shelf-life of [redacted] months has been proposed for the tetanus bulk when it is stored in stainless containers at [redacted]±[redacted]°C. The long-term stability study will be continued up to [redacted] months.

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, ≤16.7 Lf of diphtheria toxoid, and ≤6.7 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 (aluminum chloride and sodium hydroxide) as an adjuvant. Each dose also contains sodium chloride, dibasic sodium phosphate hydrate, sodium dihydrogenphosphate, M199 (Ca, Mg, phosphate, phenol red-free), dextrose, L-lysine hydrochloride, disodium edetate hydrate, and formalin as excipients. The drug product is available in glass syringes.

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

The bulk purified diphtheria toxoid and the bulk purified tetanus toxoid are diluted then sterile-filtered (pore size [redacted] μm), and then added with aluminum chloride solution and sodium hydroxide solution to form adsorbed intermediates, i.e. the bulk adsorbed diphtheria toxoid and the bulk adsorbed tetanus toxoid, respectively. Monovalent bulks of types 1, 2, and 3 are diluted and blended, and then sterile-filtered to form an intermediate, i.e. a trivalent bulk. Controls of these three intermediates as critical intermediates have been established. The bulk of purified pertussis vaccine, bulk adsorbed diphtheria toxoid, bulk adsorbed tetanus toxoid, and [redacted]-fold diluted and sterile-filtered trivalent bulk are diluted with sterile-filtered saline and blended, and then pH-adjusted to produce the final bulk. The final bulk is filled into syringes accompanied by autoclaved needles.

The following steps have been defined as critical steps: (a) The bulk adsorbed diphtheria toxoid preparation step, (b) the bulk adsorbed tetanus toxoid preparation step, (c) the trivalent bulk preparation step, (d) the final bulk preparation step, and (e) the filling step. As in-process tests, sterility test is performed at (a) and (b), test for diphtheria antigen content (ELISA) is performed at (a), test for tetanus antigen content (ELISA) is performed at (b), test for aluminum content is performed at (a) and (b), test for D-antigen content is performed at (c), and filter integrity test is performed at (a), (b), and (d).

Process validation (control of operational parameters of critical steps and quality control tests of critical intermediates) was performed on three commercial scale lots each of the bulk adsorbed diphtheria toxoid and the bulk adsorbed tetanus toxoid and three pilot scale lots of the final product. The results demonstrated the robustness of the manufacturing process and the consistency of the quality of critical intermediates. Although there were differences in the particle size according to the type of antigen, lot-to-lot consistency was demonstrated for the same antigen.

2.A.(5).3) Manufacturing process development

During product development, changes in the manufacturing process occurred as shown in Table 2-16. Based on the results of test for antigen content, test for protein nitrogen content, absorbance measurement, and quality control tests of the samples before and after sterile filtration, the pre- and post-change products were determined to be comparable.

Table 2-16. History of drug product manufacturing process changes

Manufacturing process	Manufacturing process 1	Manufacturing process 2	Manufacturing process 3
Bulk adsorbed diphtheria toxoid preparation step	Without sterile filtration	With sterile filtration	
Bulk adsorbed tetanus toxoid preparation step	Without sterile filtration	With sterile filtration	

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 2-17).

Table 2-17. 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	≤ 16.7 Lf	≤ 16.7 Lf		
Tetanus toxoid	≤ 6.7 Lf	≤ 6.7 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).4) Specifications

The specifications for the drug product include description, test for pH, sterility test, bacterial endotoxins test, test for extractable volume, foreign insoluble matter test, insoluble particulate matter test, test for freedom from abnormal toxicity, mouse body weight decreasing toxicity test, mouse histamine sensitization test, test for detoxification of diphtheria toxin, test for detoxification of tetanus toxin, test for aluminum content, test for formaldehyde content, test for D-antigen content, test for protein nitrogen content, potency test (pertussis), potency test (diphtheria), potency test (tetanus), potency test (inactivated polio), test for osmotic pressure ratio, uniformity of dosage unit tests, and identity test. The uniformity of dosage unit tests have been included in the specifications in the course of the regulatory review.

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

In addition to the standards and reference materials 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]) are used in the specification tests for the drug product. These standards are distributed by NIID and stored at ≤[redacted]°C. The Reference Inactivated Polio Vaccine (Sabin strain) to be used in the potency test (inactivated polio) is also distributed as the standard by NIID and stored at ≤[redacted]°C. The standard viruses for the determination of types 1, 2, and 3 poliovirus D-antigen to be used in the test for D-antigen content are purchased as reference materials from the Japan Poliomyelitis Research Institute and stored at ≤[redacted]°C.

As in-house reference materials, the bulk purified diphtheria toxoid and the bulk purified tetanus toxoid produced at a commercial scale are diluted and used in the test for diphtheria antigen content (ELISA) and the test for tetanus antigen content (ELISA), respectively. Each in-house reference material is characterized by protein nitrogen content, calibration curve analysis for antigen content determination, SDS-PAGE electrophoresis, and gel filtration chromatographic pattern and stored at ≤[redacted]°C. For renewal of in-house reference materials, the following points will be checked: the slopes of the calibration curves for the old and new reference materials (mean ± [redacted]SD) are the same; and when the old and new reference materials are used for determining the antigen content of the same sample, the measurements (mean ± [redacted]SD) agree with each other.

2.A.(5).6) Stability of drug product

Stability studies on the drug product are as shown in Table 2-18.

Table 2-18. Stability studies on drug product

Study		No. of lots	Storage condition	Storage package	Storage period
Long term		3	10 ± 1°C, protected from light	Glass syringe	24 months[a)]
Accelerated (1)		3	25 ± 2°C, protected from light		6 months
Accelerated (2)[b)]					[redacted] weeks
Stress	Temperature (1)	1	[redacted]°C, protected from light		[redacted] days
	Temperature (2)[b)]	3			[redacted] hours
	Light (1)	1	[redacted]°C, light providing an overall illumination of not less than 1.2 million lux·hr and an integrated near ultraviolet energy of not less than 200 W·hr/m^2	Glass syringe, protected from light by aluminum foil	–
	Light (2)[b)]	1		Glass syringe + secondary package, protected from light by aluminum foil	–

a) The study is ongoing.
b) The attributes tested were those in which changes had been observed in Study (1).

At the long-term storage condition, [redacted] increased at [redacted] months of storage and [redacted] in [redacted] test at [redacted] months of storage, which were the changes observed also with the approved DPT, but there were no changes over time for other attributes tested. At the accelerated condition, in addition to the changes noted in the long-term stability study, [redacted], [redacted] etc. were observed. In the stress study (light), changes such as [redacted] were reduced when the samples were protected from light.

Based on the above, a shelf life of 24 months has been proposed for the drug product when it is stored in glass syringes, protected from light, at ≤10°C (avoid freezing). The long-term stability study will be continued up to [redacted] months.

2.B Outline of the review by PMDA

Although PMDA is currently asking the applicant to explain the details of the manufacturing process and controls etc. for the product, 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, challenge/protection studies with *Bordetella pertussis* and tetanus toxin and immunogenicity studies were conducted using Formulation H, Formulation M, and Formulation L [see "2.A.(5).3) Manufacturing process development"].

Safety pharmacology studies with Formulation H were conducted to assess its effects on the central nervous system, cardiovascular and respiratory systems.

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

3.(i).A.(1).1) Challenge/protection studies with *Bordetella pertussis* and tetanus toxin in mice (4.2.1.1.1, Study [redacted]23)

Mice (19-20 females/group) were immunized with a single intraperitoneal injection of 0.5 mL of Formulation M diluted at 1:200, 1:40, or 1:8 and challenged intracerebroventricularly with *Bordetella pertussis* 3 weeks later and this experiment was repeated nine times (a total of 539 mice in three groups). In the nine experiments, the proportion of mice that did not have any of the symptoms caused by *Bordetella pertussis*, e.g. paralysis and parietal swelling at 14 days after the injection of *Bordetella pertussis* was 0% to 15% for 1:200 dilution, 15% to 30% for 1:40 dilution, and 50% to 75% for 1:8 dilution.

Mice (10 females/group) were immunized with a single subcutaneous injection in the abdominal wall of 0.5 mL of Formulation M diluted at 1:800, 1:400, 1:200, or 1:100 and challenged with tetanus toxin by subcutaneous injection in the thigh 4 weeks later and this experiement was repeated nine times (a total of 360 mice in four groups). In the nine experiments, the mouse survival rate at 4 days after the injection of tetanus toxin was 0% for 1:800 dilution, 0% to 30% for 1:400 dilution, 20% to 70% for 1:200 dilution, and 50% to 80% for 1:100 dilution.

Based on the above, it was discussed that Formulation M is protective against *Bordetella pertussis* and tetanus toxin.

3.(i).A.(1).2) Inactivated poliovirus immunogenicity study in cynomolgus monkeys (4.2.1.1.2, Study [redacted]36)

Cynomolgus monkeys (3 males/group) were injected subcutaneously on the back with 0.5 mL of Formulation H, Formulation M, or Formulation L (a total of 9 cynomolgus monkeys in three groups) at Weeks 0, 3, 6, and 17. Neutralizing antibody titers against attenuated and virulent strains of poliovirus types 1, 2, and 3 at Week 9 (at 3 weeks after the third dose) and Week 20 (at 3 weeks after the fourth dose) were as shown in Table 3-1.

Table 3-1. Mean neutralizing antibody titers (log_2) at Weeks 9 and 20

		Attenuated strains of poliovirus			Virulent strains of poliovirus		
		Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Week 9	Formulation H	10.6	11.1	9.5	8.3	10.6	8.3
	Formulation M	9.8	12.0	11.5	8.3	9.6	10.3
	Formulation L	7.1	7.6	6.8	5.5	4.3	4.8
Week 20	Formulation H	13.3	14.0	13.5	10.8	13.8	13.3
	Formulation M	11.6	13.8	13.1	8.6	12.8	13.0
	Formulation L	9.5	11.5	11.1	8.6	9.0	10.1

As a result, cynomolgus monkeys in all groups had neutralizing antibody titers of $\geq 2^{4.3}$ against attenuated or virulent strains of poliovirus, and had higher neutralizing antibody titers after the fourth dose than after the third dose. There were no major differences in the mean neutralizing antibody titers induced between Formulations H and M.

At the same time, antibody titers against diphtheria toxin and tetanus toxoid were also measured and there were no major differences in antibody titers among Formulations H, M, and L. Therefore, it was discussed that differences in inactivated poliovirus antigen contents do not affect the immunogenicity of diphtheria toxoid or tetanus toxoid.

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

3.(i).A.(2).1) Effects on central nervous system (4.2.1.3.1, Study [redacted]37)

Rats (6 males/group) received a single subcutaneous injection of 0.5 mL/kg or 1.0 mL/kg of Formulation H or saline (a total of 18 rats in three groups) and general activity and behaviour were assessed by modified Irwin's test at pre-dose and 0.5, 1, 2, 4, 8, and 24 hours post-dose. As a result, no effects on the central nervous system were observed. When 0.5 mL/kg of Formulation H was administered, the doses of the *Bordetella pertussis* protective antigen, diphtheria toxoid, and tetanus toxoid were approximately 6 times the proposed clinical doses and the doses of inactivated poliovirus types 1, 2, and 3 were approximately 12 times the proposed clinical doses.

3.(i).A.(2).2) Effects on cardiovascular and respiratory systems (4.2.1.3.2, Study [redacted]38)

Cynomolgus monkeys (4 males/group) received a single subcutaneous injection of 0.5 mL/kg or 1.0 mL/kg of Formulation H or saline (a total of 8 cynomolgus monkeys in two groups). Blood pressure, heart rate, ECG, and respiratory rate were measured using telemetry at pre-dose and 0.5, 1, 2, 4, 6, 8, and 24 hours post-dose. Blood gases (O_2 partial pressure, CO_2 partial pressure, pH, hemoglobin oxygen saturation) were also measured at pre-dose and 2, 6, and 24 hours post-dose. As a result, there were no effects on the cardiovascular or respiratory system.

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

A primary pharmacodynamic study 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.

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, 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).3) Manufacturing process development"].

3.(iii).A.(1) Single-dose toxicity (4.2.3.1.1, Study [redacted]58; 4.2.3.1.2, Study [redacted]57)

Rats (5 rats/sex/group) were subcutaneously injected with saline or 5 mL/kg or 10 mL/kg of Formulation H (a total of 30 rats in three groups) and cynomolgus monkeys (1 cynomolgus monkey/sex/group) were subcutaneously injected with 2.5 mL/kg or 5 mL/kg of Formulation H (a total of 4 cynomolgus monkeys in two groups). As a result, no deaths occurred in any group and the approximate lethal dose was considered to be >10 mL/kg in rats and >5 mL/kg in cynomolgus monkeys. When 5 mL/kg of Formulation H was administered, the doses of the *Bordetella pertussis* protective antigen, diphtheria toxoid, and tetanus toxoid were approximately 60 times the proposed clinical doses and the doses of inactivated poliovirus types 1, 2, and 3 were approximately 120 times the proposed clinical doses.

3.(iii).A.(2) Repeat-dose toxicity (4.2.3.2.1, Study [redacted]56; 4.2.3.2.2, Study [redacted]55)

Rats (10 rats/sex/group) received five doses of saline or 0.5 mL/kg or 1 mL/kg of Formulation H (a total of 60 rats in three groups) by subcutaneous injection at weekly intervals. As a result, pathological findings included mononuclear cell infiltration and white nodules or red lesions

representing granulomas at the injection site in the Formulation H 0.5 mL/kg and 1.0 mL/kg groups. Hematology findings included high eosinophil count and high monocyte count in females in both Formulation H groups. Blood biochemistry findings included low A/G ratio and a low percentage of albumin in all groups and a high percentage of β-globulin or γ-globulin in females in the Formulation H groups.

Cynomolgus monkeys (3 cynomolgus monkeys/sex/group) received five doses of saline, 0.5 mL/kg, or 1 mL/kg of Formulation H (a total of 18 cynomolgus monkeys in three groups) by subcutaneous injection at weekly intervals. As a result, pathological findings included inflammatory cell infiltration, residual test article like-material, and white nodules or red lesions representing granulomas, etc. at the injection site in the Formulation H 0.5 mL/kg and 1.0 mL/kg groups. In the Formulation H 1 mL/kg group, high white blood cell count and high lymphocyte count in males, increased spleen weights in females, and enlargement of germinal centers in the spleen in males and females were observed.

Based on the above, the no observed adverse effect level (NOAEL) except for effects at the injection site was considered to be 1 mL/kg.

3.(iii).A.(3) Genotoxicity

No genotoxicity studies have been conducted.

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

No carcinogenicity studies have been conducted.

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

No reproductive and developmental toxicity studies have been conducted. Histopathologic examinations in the repeat-dose toxicity studies revealed no effects on male and female reproductive organs.

3.(iii).A.(6) Local tolerance (4.2.3.6.1, Study [redacted]39; 4.2.3.6.2, Study [redacted]85)

A local irritation study in which rabbits (6 males/group) received a single intramuscular injection of 0.5 mL of test article into the vastus lateralis muscle was conducted. Rabbits were assigned to receive Formulation H, Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine (DPT), 0.75 vol % acetic acid solution, or 6.0 vol % acetic acid solution (a total of 24 rabbits in four groups). As a result, signs of local irritation including cell infiltration, edema, and necrosis in the Formulation H group were similar in severity to those in the DPT group.

A cumulative irritation study in which rabbits (4 rabbits/sex/group) received four doses of 0.5 mL of test article by subcutaneous injection at the same site on the back at 3-week intervals was conducted. Rabbits were assigned to receive Formulation M or DPT (a total of 16 rabbits in two groups). As a result, signs of cumulative irritation including cell infiltration, edema, accumulation of eosinophilic/basophilic material, granuloma, and hemorrhage in the Formulation M group were similar in severity to those in the DPT group.

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

PMDA concluded that there is no particular problem with the toxicity of the candidate DPT-sIPV vaccine.

4. Clinical data

4.A Summary of the submitted data

As the efficacy and safety evaluation data, the results from 4 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 and route of administration	Immunization schedule
I	332P1	Randomized double-blind	Healthy adult male subjects (20-40 years)	Formulation H group: 10 Control group: 10 (DPT)	0.5 mL/ Subcutaneous	A single dose
II	332P2	Randomized double-blind	Healthy children (3 to < 7 months of age at the first dose)	Formulation H group: 42 Control group: 43 (DPT + OPV)	0.5 mL/ Subcutaneous	・Formulation H or DPT Primary immunization: three doses at 3- to 8-week intervals Booster immunization: a single dose 6-18 months after the primary immunization ・OPV Two doses at least 6 weeks apart between 4-8 weeks after the primary immunization with DPT and up to 5 weeks prior to booster immunization
II	332P2b	Randomized double-blind	Healthy children (3 to < 90 months of age)	Formulation H group: 33 Formulation M group: 38 Formulation L group: 33	0.5 mL/ Subcutaneous	Primary immunization: three doses at intervals of 20-56 days Booster immunization: a single dose 6-18 months after the primary immunization
III	332P3[a)]	Randomized double-blind	Healthy children (3 to < 90 months of age)	DPT-sIPV group: 221 (DPT-sIPV[a)] + OPV placebo) Control group: 121 (DPT[a)] + OPV)	0.5 mL/ Subcutaneous	・DPT-sIPV or DPT Primary immunization: three doses at intervals of 20-56 days Booster immunization: a single dose 6-18 months after the primary immunization ・OPV or OPV placebo Two doses at least 41 days apart between 28-42 days after the primary immunization with DPT-sIPV or DPT and up to 35 days 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) was only 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	≤ 16.7 Lf	≤ 16.7 Lf		
Tetanus toxoid	≤ 6.7 Lf	≤ 6.7 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 332P1; Study period, [redacted] 2007 to [redacted] 20[redacted])

A randomized, double-blind, parallel-group, comparative study in healthy adult male subjects aged between 20 and 40 (Target sample size of 20; 10 subjects in the Formulation H group, 10 subjects in the control group) was conducted at a single center in Japan to evaluate the safety of Formulation H (Table 4-2) (Formulation H group) vs. Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine (DPT) (control group). A single dose of 0.5 mL of Formulation H or DPT was to be given by subcutaneous injection.

In the study, subjects were stratified by anti-diphtheria toxoid antibody titer at screening at randomization. Twenty enrolled subjects (10 subjects per group) 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 28-day period following study vaccination (starting from the following day of vaccination; the same rule applies hereinafter) was 30.0% (3 of 10 subjects) in the Formulation H group and 80.0% (8 of 10 subjects) in the control 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. The percentage of subjects who had at least one adverse reaction was the same as that for adverse events, i.e. 30.0% (3 of 10 subjects) in the Formulation H group and 80.0% (8 of 10 subjects) in the control group. Adverse reactions reported by at least 2 subjects in the Formulation H group or the control group were as shown in Table 4-3.

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

Adverse reaction	Formulation H group (N = 10)		Control group (N = 10)	
	n	%	n	%
Injection site erythema	2	20.0	4	40.0
Injection site pain	0	0	4	40.0
Injection site pruritus	0	0	2	20.0
Injection site warmth	0	0	2	20.0
Injection site swelling	1	10.0	2	20.0

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 332P2; Study period, [redacted] 20[redacted] to [redacted] 20[redacted])

A multicenter, randomized, double-blind, parallel-group, comparative study in healthy children 3 to <7 months of age at the first dose (Target sample size of 68; 34 subjects in the Formulation H group, 34 subjects in the control group) was conducted at 6 centers in Japan to evaluate the safety and immunogenicity of Formulation H (Table 4-2) compared with the control (DPT plus Live Oral Poliomyelitis Vaccine [OPV]).

Subjects were to receive a total of four doses of 0.5 mL of Formulation H or DPT by subcutaneous injection: three doses at 3- to 8-week intervals (primary immunization) and a single dose 6 to 18 months after the third dose (booster immunization). In addition, subjects in the control group were to receive two oral doses of 0.05 mL of OPV at least 6 weeks apart between 4 to 8 weeks after the third dose of DPT and up to 5 weeks prior to the fourth dose of DPT.

A total of 85 subjects (42 subjects in the Formulation H group, 43 subjects in the control group) were enrolled into the study, all of whom were included both in the safety analysis population and in the efficacy analysis population.

In the study, the blind was to be broken after the safety and immunogenicity data were frozen after the third dose (4-8 weeks after the third dose in Study 332P2) and subsequent assessments were to be performed in an unblinded manner. For immunogenicity evaluation, antibody titers were measured prior to the first dose of Formulation H or DPT, after the third dose of Formulation H or DPT, prior to the fourth dose of Formulation H or DPT, and after the fourth dose of Formulation H or DPT (4-8 weeks after the fourth dose in Study 332P2), and after the second dose of OPV in the control group (5-8 weeks after the second dose in Study 332P2).

In the efficacy analysis population, the seropositivity rates for neutralizing antibodies against attenuated and virulent strains of poliovirus types 1, 2, and 3 after the third dose of Formulation H (the percentages of subjects with neutralizing antibody titers of ≥1:4 in Study 332P2) and

their 95% confidence intervals were all 100% (42 of 42 subjects) [91.6, 100]. On the other hand, the neutralizing antibody seropositivity rates after the second dose of OPV in the control group and their 95% confidence intervals were 100% (43 of 43 subjects) [91.8, 100] for the attenuated strains of poliovirus types 1 and 2, 93.0% (40 of 43 subjects) [80.9, 98.5] for the attenuated strain of poliovirus type 3, 100% (42 of 42 subjects) [91.6, 100] for the virulent strains of poliovirus types 1 and 2, and 92.9% (39 of 42 subjects) [80.5, 98.5] for the virulent strain of poliovirus type 3.

The mean neutralizing antibody titers against attenuated and virulent strains of poliovirus types 1, 2, and 3 (log_2) in the Formulation H and control groups are shown in Table 4-4.

Table 4-4. Mean neutralizing antibody titers against poliovirus (log_2)[a] (Efficacy analysis population)

Formulation H group		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)
		N = 42	N = 42	N = 42	N = 42
Attenuated strains	Type 1	2.76 (1.94)	11.27 (1.97)	9.26 (2.01)	12.10 (1.79)
	Type 2	2.46 (1.38)	11.01 (1.07)	9.83 (1.20)	13.44 (0.93)
	Type 3	1.19 (0.60)	11.21 (1.33)	8.30 (1.78)	13.13 (1.46)
		N = 42	N = 42	N = 42	N = 42
Virulent strains	Type 1	1.45 (1.04)	7.30 (1.45)	5.67 (1.66)	8.46 (1.27)
	Type 2	2.27 (1.49)	10.31 (1.33)	9.62 (1.53)	13.26 (1.25)
	Type 3	1.10 (0.35)	10.42 (1.37)	7.77 (1.86)	12.25 (1.47)
Control group		Prior to the first dose	After the second dose of OPV	Prior to the fourth dose[b]	After the fourth dose[b]
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
		N = 43	N = 43	N = 43	N = 43
Attenuated strains	Type 1	2.73 (2.60)	12.01 (2.10)	11.59 (2.03)	11.23 (1.93)
	Type 2	2.14 (1.44)	11.99 (1.83)	11.00 (2.21)	10.43 (1.97)
	Type 3	1.17 (0.41)	8.09 (2.76)	7.67 (2.69)	7.37 (2.74)
		N = 42	N = 42	N = 42	N = 43
Virulent strains	Type 1	1.57 (1.51)	8.83 (2.30)	8.36 (2.36)	8.07 (2.30)
	Type 2	2.10 (1.45)	11.70 (2.07)	10.77 (2.32)	10.28 (2.11)
	Type 3	1.02 (0.15)	7.23 (2.75)	6.85 (2.58)	6.74 (2.85)

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) below the lower limit of quantitation (2.0) were to be treated as one-half of the lower limit of quantitation.
b) As the OPV vaccination is completed with two doses, the data are not intended to show if subjects had booster responses.

Regarding safety, the incidence of adverse events in the 28-day period following vaccination with Formulation H or DPT (the period up to the next dose if the interval between the first and second doses or between the second and third doses was shorter than 28 days) was 97.6% (41 of 42 subjects) in the Formulation H group and 100% (43 of 43 subjects) in the control group. The incidence of adverse reactions was 76.2% (32 of 42 subjects) in the Formulation H group and 74.4% (32 of 43 subjects) in the control group. Adverse events and/or adverse reactions that were reported by at least 10% of subjects in either study 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 either group
(Safety analysis population)

		Formulation H group (N = 42)				Control group (N = 43)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%
Local injection site reactions	Injection site erythema	21	50.0	21	50.0	27	62.8	27	62.8
	Injection site induration	10	23.8	10	23.8	19	44.2	19	44.2
	Injection site swelling	10	23.8	10	23.8	15	34.9	15	34.9
Systemic reactions	Pyrexia	34	81.0	12	28.6	36	83.7	13	30.2
	Diarrhoea	28	66.7	6	14.3	24	55.8	6	14.0
	Rhinorrhoea	28	66.7	0	0	23	53.5	1	2.3
	Mood altered	25	59.5	9	21.4	20	46.5	3	7.0
	Cough	24	57.1	1	2.4	24	55.8	0	0
	Anorexia	21	50.0	5	11.9	20	46.5	1	2.3
	Rash	11	26.2	1	2.4	14	32.6	3	7.0
	Vomiting	10	23.8	2	4.8	16	37.2	0	0
	Dermatitis diaper	10	23.8	0	0	15	34.9	0	0
	Arthropod sting	9	21.4	0	0	13	30.2	0	0
	Pharyngeal erythema	9	21.4	0	0	10	23.3	0	0
	Heat rash	8	19.0	0	0	14	32.6	0	0
	Eczema infantile	7	16.7	0	0	3	7.0	0	0
	Nasal congestion	6	14.3	0	0	5	11.6	0	0
	Eye discharge	5	11.9	0	0	14	32.6	0	0
	Wheezing	2	4.8	0	0	5	11.6	0	0
	Eczema	2	4.8	0	0	5	11.6	1	2.3

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

Serious adverse events in the 28-day period following each vaccination with Formulation H or DPT or in the 35-day period following each vaccination with OPV were febrile convulsion reported by 1 subject in the Formulation H group, gastroenteritis rotavirus reported by 1 subject in the control group following vaccination with DPT, and bacteraemia and febrile convulsion reported by 1 subject in the control group following vaccination with OPV, 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 II clinical study (5.3.5.1.3, Study 332P2b; 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 90; 30 subjects per group) was conducted at 16 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 intervals of 20 to 56 days (primary immunization) and a single dose 6 to 18 months after the third dose (booster immunization).

A total of 104 subjects (33 subjects in the Formulation H group, 38 subjects in the Formulation M group, 33 subjects in the Formulation L group) were enrolled into the study. All of the enrolled subjects were included in the safety analysis population and in the Full Analysis Set (FAS) for primary immunization and 102 subjects (32 subjects in the Formulation H group, 38 subjects in the Formulation M group, 32 subjects in the Formulation L group) were included in the FAS for booster immunization, which was used for the primary analysis for immunogenicity. Excluded were 1 subject in the Formulation H group and 1 subject in the Formulation L group who did not receive the fourth dose of study vaccine (consent withdrawal [1 subject], moving out of the area [1 subject]).

In the study, the blind was to be broken after the immunogenicity and safety data were frozen after the third dose (28-42 days after the third dose in Study 332P2b) and subsequent assessments were to be performed in an unblinded manner. Based on the immunogenicity and safety data after the first three doses, the doses of inactivated poliovirus antigens to be used for a phase III clinical study were determined [see "4.B.(5).1) Doses of antigens"].

For immunogenicity evaluation, the seropositivity rates for neutralizing antibodies against attenuated and virulent strains of poliovirus types 1, 2, and 3 (the percentages of subjects with neutralizing antibody titers of ≥1:8) after the fourth dose of Formulation H, Formulation M, or Formulation L are shown in Table 4-6.

Table 4-6. Seropositivity rates for neutralizing antibodies against poliovirus after the fourth dose (FAS for booster immunization)

		Formulation H group (N = 32)		Formulation M group (N = 38)		Formulation L group (N = 32)	
		n/N	% [95% CI]	n/N	% [95% CI]	n/N	% [95% CI]
Attenuated strains	Type 1	32/32	100 [89.1, 100]	38/38	100 [90.7, 100]	32/32	100 [89.1, 100]
	Type 2	32/32	100 [89.1, 100]	38/38	100 [90.7, 100]	32/32	100 [89.1, 100]
	Type 3	32/32	100 [89.1, 100]	38/38	100 [90.7, 100]	32/32	100 [89.1, 100]
Virulent strains	Type 1	32/32	100 [89.1, 100]	38/38	100 [90.7, 100]	32/32	100 [89.1, 100]
	Type 2	32/32	100 [89.1, 100]	38/38	100 [90.7, 100]	32/32	100 [89.1, 100]
	Type 3	32/32	100 [89.1, 100]	38/38	100 [90.7, 100]	32/32	100 [89.1, 100]

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

The safety observation period was 27 days from each dose (the period up to the next dose if the interval between the first and second doses or between the second and third doses was shorter than 27 days). The incidence of adverse events was 100% (33 of 33 subjects) in the Formulation H group, 100% (38 of 38 subjects) in the Formulation M group, and 100% (33 of 33 subjects) in the Formulation L group and the incidence of adverse reactions was 100% (33 of 33 subjects) in the Formulation H group, 92.1% (35 of 38 subjects) in the Formulation M group, and 97.0% (32

of 33 subjects) in the Formulation L group. Adverse events and/or adverse reaction that were reported by at least 10% of subjects in any study group are shown in Table 4-7.

Table 4-7. 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 = 33)				Formulation M group (N = 38)				Formulation L group (N = 33)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%	n	%	n	%
Local injection site reactions	Injection site erythema	29	87.9	29	87.9	28	73.7	28	73.7	24	72.7	24	72.7
	Injection site induration	27	81.8	27	81.8	20	52.6	20	52.6	23	69.7	23	69.7
	Injection site swelling	11	33.3	11	33.3	11	28.9	11	28.9	16	48.5	16	48.5
	Injection site haematoma	2	6.1	2	6.1	5	13.2	5	13.2	1	3.0	1	3.0
Systemic reactions	Pyrexia	31	93.9	21	63.6	35	92.1	18	47.4	32	97.0	16	48.5
	Rhinorrhoea	25	75.8	5	15.2	29	76.3	2	5.3	28	84.8	8	24.2
	Cough	25	75.8	5	15.2	27	71.1	2	5.3	25	75.8	2	6.1
	Diarrhoea	18	54.5	8	24.2	22	57.9	12	31.6	24	72.7	7	21.2
	Mood altered	23	69.7	12	36.4	21	55.3	10	26.3	18	54.5	7	21.2
	Rash	18	54.5	3	9.1	17	44.7	4	10.5	13	39.4	1	3.0
	Pharyngeal erythema	11	33.3	2	6.1	15	39.5	4	10.5	12	36.4	2	6.1
	Heat rash	8	24.2	1	3.0	15	39.5	2	5.3	10	30.3	1	3.0
	Anorexia	13	39.4	3	9.1	14	36.8	5	13.2	13	39.4	6	18.2
	Vomiting	10	30.3	3	9.1	14	36.8	2	5.3	15	45.5	4	12.1
	Dermatitis diaper	5	15.2	0	0	14	36.8	0	0	10	30.3	0	0
	Eye discharge	9	27.3	2	6.1	10	26.3	1	2.6	5	15.2	0	0
	Nasal congestion	7	21.2	2	6.1	7	18.4	0	0	6	18.2	1	3.0
	Sneezing	5	15.2	0	0	7	18.4	0	0	5	15.2	0	0
	Arthropod sting	5	15.2	0	0	7	18.4	0	0	3	9.1	0	0
	Productive cough	4	12.1	1	3.0	6	15.8	0	0	6	18.2	2	6.1
	Wheezing	1	3.0	0	0	5	13.2	1	2.6	6	18.2	0	0
	Asthenia	2	6.1	0	0	5	13.2	0	0	3	9.1	0	0
	Eczema	6	18.2	3	9.1	4	10.5	1	2.6	9	27.3	4	12.1
	Dry skin	0	0	0	0	4	10.5	0	0	0	0	0	0
	Erythema	5	15.2	1	3.0	3	7.9	0	0	2	6.1	0	0
	Otorrhoea	4	12.1	0	0	3	7.9	0	0	0	0	0	0
	Decreased appetite	6	18.2	1	3.0	2	5.3	0	0	6	18.2	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 2 events reported by 2 subjects in the Formulation M group (exanthema subitum, gastroenteritis) and 6 events reported by 2 subjects in the Formulation L group (bronchopneumonia [2], urinary tract infection [2], pneumonia [1], exanthema subitum [1]), 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.(4) Japanese phase III clinical study (5.3.5.1.4, Study 332P3; 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 315; 210 subjects in the DPT-sIPV group, 105 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 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 intervals of 20 to 56 days (primary immunization) and a single dose 6 to 18 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 41 days apart between 28 to 42 days after the third dose of DPT-sIPV or DPT and up to 35 days 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 342 subjects (221 subjects in the DPT-sIPV group, 121 subjects in the control group) were enrolled into the study. All of the enrolled subjects were included in the safety analysis population and 341 subjects (221 subjects in the DPT-sIPV group, 120 subjects in the control group) were included in the Full Analysis Set (FAS), which was used for the primary analysis for immunogenicity.Excuded was 1 subject without post-vaccination immunogenicity data due to moving out of the area. From the FAS, 336 subjects (217 subjects in the DPT-sIPV group, 119 subjects in the control group) were included in the Per Protocol Set (PPS), which was used for the analyses for the secondary objective. Excluded were 5 subjects with major protocol deviations, e.g. violation as to dosing interval.

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 (28-42 days after the third dose in Study 332P3), prior to the fourth dose of DPT-sIPV or DPT, and after the fourth dose of DPT-sIPV or DPT (28-42 days after the fourth dose in Study 332P3).

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, which were all 100% (221 of 221 subjects) and a one-sample binomial test rejected the null hypothesis (antibody

seropositivity rates ≤90%) (P <0.001) and showed that the neutralizing antibody seropositivity rates exceed 90% (Table 4-8).

Table 4-8. Seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus after the third dose of DPT-sIPV (FAS)

		DPT-sIPV group (N = 221)		
		n/N	% [95% CI]	*P*-value[a]
Attenuated strains	Type 1	221/221	100 [98.3, 100]	< 0.001
	Type 2	221/221	100 [98.3, 100]	< 0.001
	Type 3	221/221	100 [98.3, 100]	< 0.001

N: No. of subjects included in the analysis population, n: No. of seropositive subjects
a) One-sample binomial test (null hypothesis: antibody seropositivity rates ≤90%), One-sided level of significance of 2.5%

Table 4-9. 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 [95% CI]	Mean [95% CI]	Mean [95% CI]	Mean [95% CI]
DPT-sIPV group	N = 221	N = 221	N = 218	N = 218
Type 1	2.94 [2.67, 3.22]	11.02 [10.78, 11.26]	9.00 [8.74, 9.27]	12.13 [11.93, 12.33]
Type 2	2.52 [2.31, 2.74]	10.48 [10.32, 10.64]	9.01 [8.81, 9.21]	12.61 [12.46, 12.77]
Type 3	1.33 [1.23, 1.44]	10.79 [10.59, 10.99]	7.83 [7.57, 8.09]	12.22 [12.03, 12.42]
Control group	N = 119	N = 120	N = 119	N = 119
Type 1	2.98 [2.61, 3.35]	2.41 [2.02, 2.79]	11.95 [11.50, 12.39]	11.55 [11.10, 12.01]
Type 2	2.53 [2.22, 2.83]	1.86 [1.57, 2.15]	9.96 [9.61, 10.31]	9.62 [9.29, 9.95]
Type 3	1.47 [1.25, 1.69]	1.38 [1.20, 1.56]	7.46 [6.87, 8.04]	7.12 [6.55, 7.69]

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) below the lower limit of quantitation (2.0) were to be treated as one-half of the lower limit of quantitation.
b) Prior to the first dose of OPV in the control group
c) After the second dose of OPV in the control group

In the FAS, the seropositivity rates for neutralizing antibodies against attenuated strains of poliovirus types 1, 2, and 3 after the fourth dose in the control group (≥9 weeks after the second dose of OPV) and their 95% confidence intervals were 97.5% (116 of 119 subjects) [92.8, 99.5], 99.2% (118 of 119 subjects) [95.4, 100], and 83.2% (99 of 119 subjects) [75.2, 89.4], respectively.

The mean neutralizing antibody titers against attenuated strains of poliovirus types 1, 2, and 3 (log_2) in the DPT-sIPV and control groups are shown in Table 4-9.

In the PPS, the seropositivity rates for antibodies against *Bordetella pertussis* (pertussis toxin [PT] and filamentous hemagglutinin [FHA]), diphtheria toxin, and tetanus toxoid after the third dose of DPT-sIPV or DPT (the percentage of subjects with positive antibody titers) and their 95% confidence intervals were 100% in both groups (217 of 217 subjects in the DPT-sIPV group, 119 of 119 subjects in the control group), except for 98.6% (214 of 217 subjects) [96.0, 99.7] for PT and 99.1% (215 of 217 subjects) [96.7, 99.9] for FHA in the DPT-sIPV group and 99.2% (118 of 119 subjects) [95.4, 100] for PT in the control group. Seropositivity was defined as ≥10 ELISA units (EU)/mL for anti-PT antibody; ≥10 EU/mL for anti-FHA antibody; ≥0.1

international units (IU)/mL for anti-diphtheria toxin antibody; and ≥0.01 IU/mL for anti-tetanus toxoid antibody.

The geometric mean antibody titers against different antigens over time in the DPT-sIPV and control groups are shown in Table 4-10.

Table 4-10. Geometric mean antibody titers[a] against *Bordetella pertussis* (PT and FHA; EU/mL), diphtheria toxin (IU/mL), and tetanus toxoid (IU/mL) (PPS)

	Prior to the first dose	After the third dose	Prior to the fourth dose	After the fourth dose
	Geometric mean antibody titer [95% CI]	Geometric mean antibody titer [95% CI]	Geometric mean antibody titer [95% CI]	Geometric mean antibody titer [95% CI]
DPT-sIPV group	N = 217	N = 217	N = 213	N = 214
PT	0.695 [0.627, 0.769]	39.0 [35.5, 42.9]	22.5 [20.0, 25.4]	196 [175, 220]
FHA	0.978 [0.827, 1.157]	62.0 [56.7, 67.7]	30.6 [27.2, 34.5]	255 [232, 279]
Diphtheria	0.00687 [0.00567, 0.00831]	1.72 [1.57, 1.89]	1.44 [1.23, 1.68]	18.0 [16.3, 19.9]
Tetanus	0.0159 [0.0129, 0.0196]	1.32 [1.18, 1.47]	1.13 [0.92, 1.38]	5.40 [4.76, 6.12]
Control group	N = 119[b]	N = 119	N = 118	N = 118
PT	0.672 [0.592, 0.762]	39.2 [34.6, 44.6]	26.2 [21.9, 31.2]	187 [163, 214]
FHA	0.870 [0.702, 1.078]	77.5 [68.1, 88.4]	35.9 [30.4, 42.6]	305 [273, 342]
Diphtheria	0.00748 [0.00581, 0.00963]	0.982 [0.858, 1.123]	1.23 [0.99, 1.53]	11.9 [10.5, 13.6]
Tetanus	0.0167 [0.0127, 0.0219]	1.27 [1.08, 1.48]	1.33 [1.02, 1.74]	4.36 [3.68, 5.17]

N: No. of subjects included in the analysis population

a) Antibody titers below the lower limit of quantitation were to be treated as one-half of the lower limit of quantitation (the lower limit of quantitation was 0.98 EU/mL for PT, 0.78 EU/mL for FHA, 0.01 IU/mL for diphtheria, 0.005 IU/mL for tetanus). Antibody titers above the upper limit of quantitation were to be treated as the upper limit value (the upper limit of quantitation was 1250 EU/mL for PT, 1000 EU/mL for FHA, and 26 IU/mL for tetanus. No upper limit of quantitation for diphtheria has been established).

b) 118 subjects were included in the analysis for diphtheria.

The safety observation period was 27 days from each dose of DPT-sIPV or DPT (the period up to the next dose if the interval between the first and second doses or between the second and third doses was shorter than 27 days), or 34 days from each dose of OPV placebo or OPV. The incidence of adverse events was 100% (221 of 221 subjects) in the DPT-sIPV group and 99.2% (120 of 121 subjects) in the control group. The incidence of adverse reactions was 92.3% (204 of 221 subjects) in the DPT-sIPV group and 90.1% (109 of 121 subjects) in the control group. Adverse events and/or adverse reactions that were reported by at least 5% of subjects in either study group are shown in Table 4-11.

Table 4-11. 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 = 221)				Control group (N = 121)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%
Injection site[a)]	Injection site erythema	151	68.3	151	68.3	79	65.3	79	65.3
	Injection site induration	115	52.0	115	52.0	67	55.4	67	55.4
	Injection site swelling	69	31.2	69	31.2	41	33.9	41	33.9
Others	Pyrexia	213	96.4	123	55.7	108	89.3	56	46.3
	Rhinorrhoea	198	89.6	41	18.6	107	88.4	23	19.0
	Diarrhoea	182	82.4	91	41.2	97	80.2	40	33.1
	Cough	179	81.0	35	15.8	102	84.3	15	12.4
	Mood altered	132	59.7	69	31.2	64	52.9	26	21.5
	Rash	110	49.8	28	12.7	50	41.3	11	9.1
	Decreased appetite	110	49.8	26	11.8	55	45.5	10	8.3
	Vomiting	105	47.5	26	11.8	65	53.7	16	13.2
	Pharyngeal erythema	95	43.0	25	11.3	43	35.5	8	6.6
	Productive cough	79	35.7	12	5.4	33	27.3	5	4.1
	Nasal congestion	65	29.4	9	4.1	22	18.2	4	3.3
	Eye discharge	63	28.5	2	0.9	23	19.0	3	2.5
	Dermatitis diaper	63	28.5	1	0.5	25	20.7	0	0
	Heat rash	58	26.2	0	0	34	28.1	0	0
	Eczema	54	24.4	9	4.1	26	21.5	5	4.1
	Wheezing	51	23.1	5	2.3	24	19.8	0	0
	Sneezing	46	20.8	4	1.8	30	24.8	4	3.3
	Arthropod sting	40	18.1	0	0	7	5.8	0	0
	Otitis media	30	13.6	1	0.5	17	14.0	1	0.8
	Erythema	21	9.5	6	2.7	7	5.8	1	0.8
	Asthenia	20	9.0	4	1.8	12	9.9	2	1.7
	Impetigo	18	8.1	0	0	8	6.6	0	0
	Insomnia	17	7.7	3	1.4	12	9.9	4	3.3
	Ocular hyperaemia	16	7.2	0	0	3	2.5	0	0
	Dry skin	15	6.8	4	1.8	4	3.3	0	0
	Eczema infantile	14	6.3	0	0	8	6.6	0	0
	Pruritus	13	5.9	3	1.4	5	4.1	1	0.8
	Constipation	13	5.9	1	0.5	6	5.0	0	0
	Dysphonia	12	5.4	1	0.5	4	3.3	0	0
	Urticaria	11	5.0	1	0.5	6	5.0	1	0.8
	Rash papular	11	5.0	0	0	5	4.1	0	0
	Dermatitis	5	2.3	0	0	9	7.4	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 11 events reported by 8 subjects in the DPT-sIPV group (gastroenteritis rotavirus [2], convulsion [1], exanthema subitum [1], pneumonia [1], otitis media acute [1], gastroenteritis viral [1], bronchopneumonia [1], meningitis bacterial [1], otitis media acute [1], pyrexia [1]) and 5 events reported by 5 subjects in the control group (pneumonia [2], bronchopneumonia [1], respiratory syncytial viral infection [1], asthma [1]), of which convulsion occurring 20 days after the first dose of DPT-sIPV in the DPT-sIPV group and pneumonia occurring 22 days after the third dose of DPT in the control

group were classified as serious adverse reactions since their 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 four clinical studies: Study 332P1, Study 332P2, Study 332P2b, and Study 332P3.

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, for the doses of inactivated poliovirus antigens, an investigation was initiated with Formulation H (Table 4-2), which was expected to show equivalent immunogenicity to an inactivated polio vaccine derived from virulent strains of poliovirus (vIPV) (which has been used widely overseas), based on rat immunogenicity studies (*Research on quality assurance of combined vaccines,* Health and Labour Sciences Research, 2004). After the tolerability and immunogenicity of Formulation H were evaluated in Studies 332P1 and 332P2, Formulation M was selected from among Formulation H, 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), based on a dose-finding study (Study 332P2b). It was decided to conduct a confirmatory study (Study 332P3) 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 (*Ann NY Acad Sci. 1995;*754:289-299, *Scand J Infect Dis*. 2007;40:247-253). Study 332P3 also evaluated the safety of DPT-sIPV and the immunogenicity of the DPT components of DPT-sIPV compared with the control group. Study 332P3 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, there were no major differences in the seropositivity rates or geometric mean antibody titers between the DPT-sIPV vaccine and the approved DPT vaccine, though these were secondary endpoint measures [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 332P3 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 (*Pediatr Infect Dis J.* 1998;17:804-809, *Vaccine.* 2001;19:825-833).

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 332P3.

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 332P3.

In Study 332P3, the neutralizing antibody seropositivity rate after the third dose of DPT-sIPV and its 95% confidence interval were 100% [98.3, 100] for all three serotypes, showing that the neutralizing antibody seropositivity rates exceed 90% (Table 4-8). Thus, 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 4-9) showed that the fourth dose of DPT-sIPV induced a booster response (about 9-fold, about 12-fold, and about 21-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 measurement, 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 4-9).

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 332P2b (Table 4-12).

Table 4-12. Mean neutralizing antibody titers against attenuated and virulent strains of poliovirus (log_2)[a] after vaccination with Formulation M (DPT-sIPV) (Study 332P2b)

	Attenuated strains (used in the production of DPT-sIPV)		Virulent strains (used in the production of foreign vIPV)	
	After the third dose[b] N = 38	After the fourth dose[c] N = 38	After the third dose[b] N = 38	After the fourth dose[c] N = 38
	Mean [95% CI]	Mean [95% CI]	Mean [95% CI]	Mean [95% CI]
Type 1	10.26 [9.73, 10.80]	12.50 [11.89, 13.11]	5.93 [5.50, 6.37]	8.33 [7.70, 8.96]
Type 2	9.79 [9.35, 10.22]	13.79 [13.27, 14.31]	9.16 [8.68, 9.63]	13.46 [12.86, 14.06]
Type 3	9.93 [9.46, 10.41]	12.75 [12.26, 13.24]	9.54 [9.07, 10.01]	12.50 [12.02, 12.98]

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) below the lower limit of quantitation (2.0) were to be treated as one-half of the lower limit of quantitation.
b) FAS for primary immunization
c) FAS for booster immunization

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 attenuated and virulent strains of poliovirus after vaccination with Formulation M (DPT-sIPV) are shown in Table 4-13. One subject was seronegative for the virulent strain of poliovirus type 1 after the third dose, but was seropositive (≥3) with a neutralizing antibody titer (log_2) of 12.5 after the fourth dose.

Table 4-13. Seropositivity rates for neutralizing antibodies against attenuated and virulent strains of poliovirus after vaccination with Formulation M (DPT-sIPV) (Study 332P2b)

		After the third dose[a]		After the fourth dose[b]	
		n/N	% [95% CI]	n/N	% [95% CI]
Attenuated strains	Type 1	38/38	100 [90.7, 100]	38/38	100 [90.7, 100]
	Type 2	38/38	100 [90.7, 100]	38/38	100 [90.7, 100]
	Type 3	38/38	100 [90.7, 100]	38/38	100 [90.7, 100]
Virulent strains	Type 1	37/38	97.4 [86.2, 99.9]	38/38	100 [90.7, 100]
	Type 2	38/38	100 [90.7, 100]	38/38	100 [90.7, 100]
	Type 3	38/38	100 [90.7, 100]	38/38	100 [90.7, 100]

N: No. of subjects included in the analysis population, n: No. of seropositive subjects
a) FAS for primary immunization, b) FAS for booster immunization

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 332P3, the cut-off values for seropositivity for anti-diphtheria toxin and anti-tetanus toxoid antibodies were defined as 0.1 IU/mL and 0.01 IU/mL, 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, *Vaccination guidance.* 13th edition; 2011: 144-164.) and the cut-off values for seropositivity for anti-PT and anti-FHA antibodies were defined as 10 EU/mL, which were the seroprotective levels estimated from convalescent antibody titers in children with whooping cough (*Journal of Pediatric Practice.* 1990;53:2275-2281). The seropositivity rates for antibodies against PT, FHA, diphtheria toxin, and tetanus toxoid after the third dose of DPT-sIPV or DPT are shown in Table 4-14. The seropositivity rates for anti-PT and anti-FHA antibodies in the DPT-sIPV group were not 100% and the difference in seropositivity rate between the DPT-sIPV and control groups and its 95% confidence interval were −0.5 [−2.8, 1.7] and −0.9 [−2.2, 0.3], respectively and there were no major differences in the seropositivity rates between the two groups. In addition, although the geometric mean antibody titer against each antigen was variable, the geometric mean antibody titers over time were similar between the two groups (Table 4-10).

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 4-14. Seropositivity rates for antibodies against *Bordetella pertussis*, diphtheria toxin, and tetanus toxoid after the third dose (Study 332P3, PPS)

	DPT-sIPV group		Control group	
	n/N	% [95% CI]	n/N	% [95% CI]
PT	214/217	98.6 [96.0, 99.7]	118/119	99.2 [95.4, 100]
FHA	215/217	99.1 [96.7, 99.9]	119/119	100 [96.9, 100]
Diphtheria	217/217	100 [98.3, 100]	119/119	100 [96.9, 100]
Tetanus	217/217	100 [98.3, 100]	119/119	100 [96.9, 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 antigen and tetanus antigen (*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 toxoid 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 in Japan as well.

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 332P3, during the 27-day post-vaccination periods following each dose of DPT-sIPV or DPT. The analysis excluded events occurring after vaccination with OPV placebo or OPV. Adverse

events and/or adverse reactions that were reported at ≥5% higher incidences in the DPT-sIPV group than in the control group were as shown in Table 4-15. Of which, only pyrexia was reported as severe (Grade 3 or 4) and there were no major differences in the incidence of Grade 3 or 4 adverse events or adverse reactions of pyrexia between the DPT-sIPV and control groups (Table 4-16). The tolerability of DPT-sIPV is considered comparable to that of the approved DPT vaccine.

Table 4-15. Adverse events and/or adverse reactions that were reported at ≥5% higher incidences in the DPT-sIPV group than in the control group (Study 332P3, Safety analysis population)

	DPT-sIPV group (N = 221)				Control group (N = 121)			
	Adverse event		Adverse reaction		Adverse event		Adverse reaction	
	n	%	n	%	n	%	n	%
Pyrexia	199	90.0	103	46.6	96	79.3	43	35.5
Mood altered	119	53.8	64	29.0	59	48.8	25	20.7
Rash	77	34.8	25	11.3	36	29.8	7	5.8
Pharyngeal erythema	76	34.4	19	8.6	30	24.8	7	5.8
Productive cough	62	28.1	8	3.6	27	22.3	4	3.3
Nasal congestion	49	22.2	8	3.6	16	13.2	3	2.5
Eye discharge	49	22.2	1	0.5	18	14.9	3	2.5
Dermatitis diaper	40	18.1	1	0.5	13	10.7	0	0
Arthropod sting	21	9.5	0	0	5	4.1	0	0
Erythema	17	7.7	6	2.7	3	2.5	1	0.8

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

Table 4-16. Incidence by maximum intensity[a)] (Study 332P3, Safety analysis population)

		DPT-sIPV group (N = 221)				Control group (N = 121)			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%
Pyrexia	Overall	199	90.0	103	46.6	96	79.3	43	35.5
	Grade 3 (≥ 39.0°C, persisted for ≤ 1 day)	53	24.0	11	5.0	28	23.1	7	5.8
	Grade 4 (≥ 39.0°C, persisted for ≥ 2 days)	45	20.4	6	2.7	28	23.1	3	2.5

N: No. of subjects included in the analysis population, n: No. of subjects with pyrexia
a) If more than one event occurred in the same subject, the maximum intensity was counted.

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 332P2, 332P2b, and 332P3 involving infants (the intended population for the vaccine) were diseases commonly seen in infants and causal relationship to DPT-sIPV was denied for all events except for 1 case of convulsion occurring 20 days after vaccination with DPT-sIPV in Study 332P3.

Based on the above, the safety profile of DPT-sIPV is tolerable. However, as febrile convulsion (non-serious; a causal relationship was denied) was also reported by two subjects after

vaccination with DPT-sIPV in Study 332P3, it is necessary to actively collect post-marketing information on convulsion and febrile convulsion.

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 was expected to show comparable immunogenicity to vIPV that has been approved overseas, 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 and virulent 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 332P2b were as shown in Table 4-17 and the seropositivity rate for neutralizing antibody against the virulent strain of poliovirus type 1 tended to be lower in the Formulation H group compared with the Formulation M and L groups.

Table 4-17. Seropositivity rates for neutralizing antibodies against poliovirus after the third dose (Study 332P2b, FAS for primary immunization)

		Formulation H group (N = 33)		Formulation M group (N = 38)		Formulation L group (N = 33)	
		n/N	% [95% CI]	n/N	% [95% CI]	n/N	% [95% CI]
Attenuated strains	Type 1	33/33	100 [89.4, 100]	38/38	100 [90.7, 100]	33/33	100 [89.4, 100]
	Type 2	33/33	100 [89.4, 100]	38/38	100 [90.7, 100]	33/33	100 [89.4, 100]
	Type 3	33/33	100 [89.4, 100]	38/38	100 [90.7, 100]	33/33	100 [89.4, 100]
Virulent strains	Type 1	30/33	90.9 [75.7, 98.1]	37/38	97.4 [86.2, 99.9]	32/33	97.0 [84.2, 99.9]
	Type 2	33/33	100 [89.4, 100]	38/38	100 [90.7, 100]	33/33	100 [89.4, 100]
	Type 3	33/33	100 [89.4, 100]	38/38	100 [90.7, 100]	33/33	100 [89.4, 100]

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

Although there were no major differences in the mean neutralizing antibody titers (log_2) among the three groups, the mean neutralizing antibody titers against attenuated and virulent strains of

poliovirus type 1 were lower in the Formulation H group and the mean neutralizing antibody titers against attenuated and virulent strains of poliovirus types 2 and 3 were lower in the Formulation L group (Table 4-18).

Table 4-18. Mean neutralizing antibody titers against poliovirus after the third dose (log_2)[a] (Study 332P2b, FAS for primary immunization)

		Formulation H group (N = 33)	Formulation M group (N = 38)	Formulation L group (N = 33)
		Mean [95% CI]	Mean [95% CI]	Mean [95% CI]
Attenuated strains	Type 1	9.94 [9.25, 10.62]	10.26 [9.73, 10.80]	10.55 [9.89, 11.20]
	Type 2	10.33 [9.88, 10.79]	9.79 [9.35, 10.22]	9.71 [9.20, 10.23]
	Type 3	9.97 [9.47, 10.47]	9.93 [9.46, 10.41]	9.14 [8.50, 9.78]
Virulent strains	Type 1	5.71 [4.97, 6.45]	5.93 [5.50, 6.37]	5.94 [5.29, 6.58]
	Type 2	9.77 [9.23, 10.31]	9.16 [8.68, 9.63]	9.11 [8.48, 9.73]
	Type 3	9.82 [9.37, 10.27]	9.54 [9.07, 10.01]	8.92 [8.33, 9.52]

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) below the lower limit of quantitation (2.0) were to be treated as one-half of the lower limit of quantitation.

Regarding safety, in Study 332P2b, there were no major differences in the incidence of adverse events or adverse reactions occurring in the periods following the first three doses among the different dose groups while the incidence of adverse events or adverse reactions of local injection site reactions increased in a dose-dependent manner (Table 4-19). The incidence of adverse reactions of pyrexia was higher in the Formulation H group than in the Formulation L and M groups (Table 4-20). Similar trends were observed also when adverse events/adverse reactions occurring in the period following the fourth dose were included in the analysis.

Table 4-19. Incidence of adverse events or adverse reactions (Study 332P2b, 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 = 33		N = 38		N = 33		N = 33		N = 38		N = 33	
Any event	33	100	37	97.4	33	100	32	97.0	33	86.8	30	90.9
Local injection site reactions	30	90.9	27	71.1	22	66.7	30	90.9	27	71.1	22	66.7
Systemic reactions[a]	33	100	37	97.4	33	100	27	81.8	27	71.1	24	72.7
After four doses	N = 33		N = 38		N = 33		N = 33		N = 38		N = 33	
Any event	33	100	38	100	33	100	33	100	35	92.1	32	97.0
Local injection site reactions	30	90.9	31	81.6	25	75.8	30	90.9	31	81.6	25	75.8
Systemic reactions[a]	33	100	33	100	33	100	29	87.9	30	78.9	27	81.8

N: No. of subjects included in the analysis population, n: No. of subjects with adverse event or adverse reaction
a) All events excluding local injection site reactions

Table 4-20. Incidence of pyrexia by maximum intensity[a] (Study 332P2b, Safety analysis population)

		Adverse event						Adverse reaction					
		Formulation H group (N = 33)		Formulation M group (N = 38)		Formulation L group (N = 33)		Formulation H group (N = 33)		Formulation M group (N = 38)		Formulation L group (N = 33)	
		n	%	n	%	n	%	n	%	n	%	n	%
Pyrexia[b]	After the first three doses												
	Overall	28	84.8	32	84.2	30	90.9	19	57.6	13	34.2	14	42.4
	Grade 3	7	21.2	7	18.4	9	27.2	1	3.0	1	2.6	2	6.1
	Grade 4	4	12.1	5	13.2	1	3.0	0	0	0	0	0	0
	After four doses												
	Overall	31	93.9	35	92.1	32	97.0	21	63.6	18	47.4	16	48.5
	Grade 3	11	33.3	10	26.3	12	36.4	1	3.0	1	2.6	3	9.1
	Grade 4	6	18.2	6	15.8	5	15.2	1	3.0	0	0	0	0

N: No. of subjects included in the analysis population, n: No. of subjects with pyrexia
a) If more than one event occurred in the same subject, the maximum intensity was counted.
b) Grade 3: ≥ 39.0°C, persisted for ≤1 day; Grade 4: ≥ 39.0°C, persisted for ≥2 days

Taking account of the immunogenicity and safety results from Study 332P2b, a confirmatory study (Study 332P3) was conducted using Formulation M. The study results 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 ≤16.7 Lf for diphtheria toxoid, ≤6.7 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 332P2, 332P2b, and 332P3 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 18 months after the primary immunization. The numbers of vaccinated subjects by dosing interval for primary and booster immunization in Study 332P3 are shown in Table 4-21. 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 (6-14 months).

Table 4-21. Numbers of vaccinated subjects by dosing interval in Study 332P3 (FAS)

Primary	Dosing interval	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
	Dose 1 to 2	103	95	14	5	4	—
	Dose 2 to 3	111	86	17	4	2	1
Booster	Dosing interval	≤ 5 months	6-8 months	9-11 months	12-14 months	15-18 months	≥ 19 months
	Dose 3 to 4	1	119	97	1	—	—

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 332P3, 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 332P3. 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, Studies 332P2b and 332P3 involving children 3 to <90 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.* 1994.), 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:

A primary series and a booster dose of DPT-sIPV are likely to be coadministered with Hib and a heptavalent pneumococcal conjugate vaccine (PCV7). Because Hib was allowed to be coadministered with DPT-sIPV in Study 332P3, the immunogenicity and safety of DPT-sIPV alone or with Hib were evaluated. As shown in Table 4-22 and Table 4-23, coadministration with Hib did not significantly affect the immunogenicity or safety of DPT-sIPV.

Table 4-22. Mean neutralizing antibody titers against attenuated strains of poliovirus (log_2)[a] in subjects who received DPT-sIPV with or without Hib (Study 332P3, FAS)

	Prior to the first dose	After the third dose	Prior to the fourth dose	After the fourth dose
	Mean [95% CI]	Mean [95% CI]	Mean [95% CI]	Mean [95% CI]
DPT-sIPV	N = 165	N = 165	N = 211	N = 211
Type 1	2.87 [2.54, 3.19]	11.12 [10.87, 11.38]	9.04 [8.77, 9.30]	12.14 [11.94, 12.34]
Type 2	2.33 [2.09, 2.58]	10.56 [10.37, 10.75]	9.02 [8.81, 9.23]	12.64 [12.48, 12.80]
Type 3	1.29 [1.17, 1.41]	10.86 [10.63, 11.10]	7.86 [7.59, 8.12]	12.24 [12.05, 12.44]
DPT-sIPV + Hib	N = 56[b]	N = 56[b]	N = 7[c]	N = 7[c]
Type 1	3.17 [2.61, 3.73]	10.72 [10.17, 11.28]	8.00 [5.43, 10.57]	11.86 [10.40, 13.31]
Type 2	3.07 [2.66, 3.48]	10.24 [9.91, 10.57]	8.71 [7.92, 9.51]	11.93 [11.03, 12.83]
Type 3	1.46 [1.23, 1.70]	10.57 [10.18, 10.96]	7.00 [5.61, 8.39]	11.64 [9.90, 13.39]

N: No. of subjects included in the analysis population
a) Neutralizing antibody titers (log_2) below the lower limit of quantitation (2.0) were to be treated as one-half of the lower limit of quantitation.
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 4-23. Adverse events and adverse reactions in subjects who received DPT-sIPV with or without Hib (Study 332P3, Safety analysis population)

	DPT-sIPV (N = 161)				DPT-sIPV + Hib[a] (N = 60)			
	Adverse event		Adverse reaction		Adverse event		Adverse reaction	
	n	%	n	%	n	%	n	%
Injection site for DPT-sIPV	119	73.9	119	73.9	40	66.7	40	66.7
Others	160	99.4	114	70.8	60	100	42	70.0

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 safety of DPT-sIPV in routine clinical settings can be determined 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, there were no particular problems. Thus, 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, 5.3.5.1.3, 5.3.5.1.4). As a result, noncompliance with the the procedures for accountability of the investigational products (the wrong investigational product was dispensed and administered to a subject) and inconsistencies between the source document and the CRF (an adverse event was

undocumented) were found at some trial sites. The sponsor was found to have failed to appropriately detect some of the above inconsistencies between the source document and the CRF during monitoring visits. 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]	Quattrovac Subcutaneous Injection Syringe
[Non-proprietary name]	Adsorbed Diphtheria-Purified Pertussis-Tetanus-Inactivated Polio (Sabin strain) Combined Vaccine
[Name of applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	January 27, 2012

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 Studies 332P2b and 332P3, 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 332P3 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 332P3, 1 subject (0.5%) had convulsion and 2 subjects (0.9%) had febrile 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, filter integrity test, sterility test, test for pH, description test, test for freedom from abnormal toxicity, test for protein content, test for formaldehyde content, immunogenicity test, test for residual live virus, and identity test were included as in-process controls at the trivalent bulk preparation step, and test for D-antigen content (supernatant after adsorption) was included in the drug product specifications. PMDA accepted these responses.

6.(1) Control of drug product

The candidate vaccine is an insoluble protein vaccine and its active ingredients are adsorbed onto aluminum gel and characteristically, the homogeneous dispersion easily becomes unstable, e.g. when the vaccine is allowed to stand, its active ingredients are precipitated. As [redacted] forms due to [redacted] of pertussis toxin (PT) and filamentous hemagglutinin (FHA), the bulk of purified pertussis vaccine has not been tested for antigen content. A characterization study has shown that the concentration of poliovirus type [redacted] inactivated antigen ([redacted] DU/mL) unadsorbed onto aluminum gel in the vaccine formulation varied from [redacted] to [redacted] DU/mL among five lots. Furthermore, testing for uniformity in the amount of each active ingredient among dosage units has not been included in the drug product specifications. Therefore, PMDA asked the applicant to explain the consistency of the vaccine filling process and the degree of uniformity in the amount of each active ingredient among dosage units.

The applicant responded as follows:

Samples were taken over time at the drug product filling process in commercial-scale production and the antigen contents of the drug product were measured (antigens: pertussis toxin [PT], filamentous hemagglutinin [FHA], diphtheria toxoid, tetanus toxoid, and inactivated poliovirus types 1, 2, and 3). As a result, the within-lot coefficient of variation was [redacted]% to [redacted]%, demonstrating that the amount of each active ingredient falls within a certain range through the filling process. As the uniformity of dosage unit tests, test for protein content and test for aluminum content will be included in the drug product specifications to check the content uniformity of individual dosage units. The test for protein content was chosen because it has a higher precision than the test for antigen content and the active ingredients as a whole can be measured. The test for aluminum content was selected to evaluate the uniformity of dosage units, considering that aluminum gel is the component most difficult to be dispersed homogeneously by agitation, during the filling process.

Based on the results of the above investigation on process control, the uniformity in the amount of each antigen among dosage units has been assured with the current filling process and PMDA accepted the applicant's explanation together with the newly proposed uniformity of dosage unit tests.

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 when stored at [redacted]°C to [redacted]°C, protected from light.

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 Quattrovac (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.

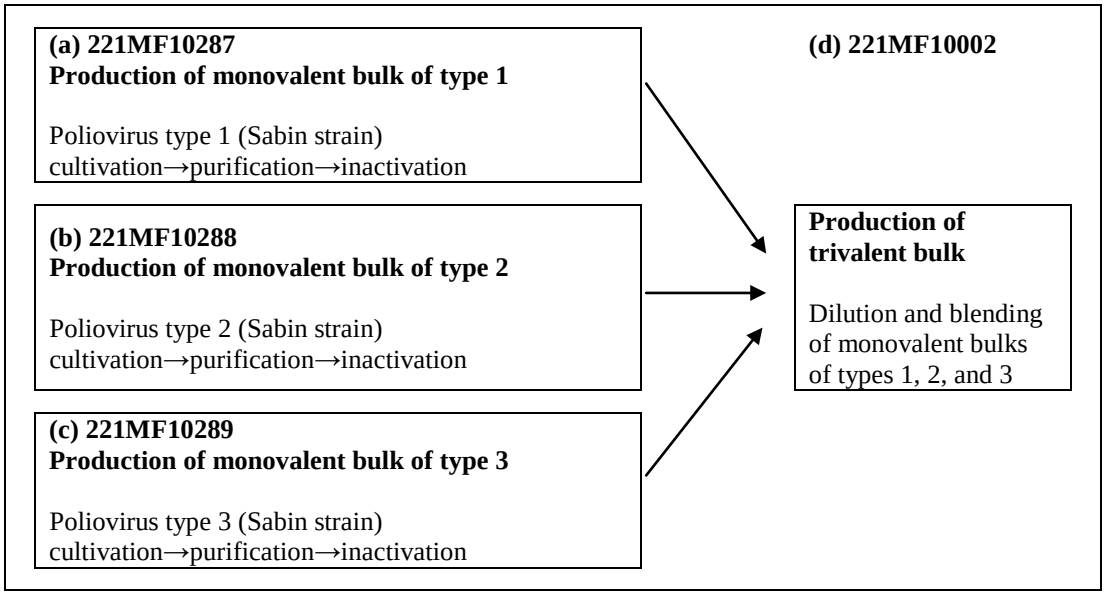

Figure 1. Information contained in MFs

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 mL mL, °C, – days		
	Expanded culture 1	L, °C, – days		
	Expanded culture 2	L, °C, – days		
	Expanded culture 3	L, °C, – days		
	Final cell culture	L, culture °C, – 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, Type 2: 10, Type 3: 10 Cultivation: L, °C, – days	Individual virus suspensions	Sterility, Mycoplasma, Virus identity
↓				
Harvest		Filtration (pore size µm→ µm→ µm)		
		Ultrafiltration (Molecular weight cutoff)	Concentrate by ultrafiltration	
↓				
Purification		Ultracentrifugation (centrifugation and treatment)		
		Anion exchange chromatography	Purified virus solution	
		Dilution Sterile filtration (pore size 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		w/v % formaldehyde °C, days th day of inactivation: Sterile filtration (pore size µm)	Inactivated virus suspension	Absence of residual live virus (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 µm)	Filtered monovalent bulk solution	
		Subdividing/Labeling	Drug substance (Monovalent bulk)	

: Critical steps or critical intermediates

Monovalent bulks of types 1, 2, and 3 (the drug substance) are diluted, blended, and sterile-filtered (µm) to form a trivalent bulk and the trivalent bulk is distributed into containers and its storage time is months at ± °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 ≤[redacted] × 10[redacted] ppm, host cell DNA was reduced to <[redacted] pg/mL, and bovine serum protein was reduced to <[redacted] ng/mL. The antibiotic level was [redacted] to [redacted] (minimum inhibitory dilution ratio) in intermediates before purification (Intermediate A and Intermediate B), but was reduced to <[redacted] 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 [redacted] cells for type 1 or in [redacted] cells for types 2 and 3, purified by [redacted], diluted in [redacted] 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 ≤[redacted]°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 Quattrovac (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 application 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.