Product Permission Document (PPD) of Haemophilus type b Conjugate Vaccine I.P. (Brand Name – Peda Hib™)

1. Introduction :-

Haemophilus Type b Conjugate Vaccine is a liquid or freeze dried preparation of polysaccharide, derived from a suitable strain of Haemophilus Influenzae type b, covalently bound to a carrier protein. The polysaccharide, polyribosylribitol phosphate, referred to as PRP, is a linear copolymer composed of repeated units of 3-β-D-ribofuronosyl-(1→1)-ribitol-5-phostphate [(C10H19O12P)n], with a defined molecular size. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

1.1 Submission file

File No. 12-40/93-DC

1.2 NDS Approval date and control

Drug/837/8324 dated 01/07/2010.

1.3 PPD –Biological revision date and control

PPD Biological Rev 02, dated 20/11/2014.

1.4 Proprietary Name

Peda Hib™

1.5 Non Proprietary name and common name of drug substance

Haemophilus type b conjugate vaccine (I.P.)

1.6 Company Name

BIO-MED (P) LTD.
C-96, Site No. 1,
Bulandshahr Road Industrial Area,
Ghaziabad - 201 009 (U.P.) INDIA
Phone : 0120-4157534, 4204862
Fax : 0120-4340219
e-Mail :bmvaccine@yahoo.com
Website: www.biomed.co.in

1.7 Name of Indian Distributer/Agent

Not Applicable as we are indigenous manufacturer of vaccine.

1.8 Therapeutic or Pharmacological classification

Vaccine/injectable

1.9 Dosage form(s)

Lyophilised vaccine

1.10 Strength (s)

Each Single dose (0.5 ml)lyophilisate contains:
10 µg polysaccharide of Haemophilus type b conjugated to 20 µg Tetanus toxoid protein.
Lactose (I.P).....2mg
Sucrose (I.P).....42.5mg
Thiomersal (I.P.)(Preservative).....0.05mg

1.11 Route of Administration

Administer 0.5 ml by intramuscular injection

1.12 Maximum Daily Dose

Not Applicable

2.0 New Active Substance (NAS) :-

Haemophilus type b conjugate vaccine is produced in all over the world for several decades. There is predefined parameter for the manufacturing of Haemophilus type b conjugate vaccine. All the products used in the production of vaccine is already known. All excipients used have been previously used for manufacture of human vaccine(s). None of the excipients are novel.

S. Drug substance (name & manufacturer)

S.1 Manufacturer (name, manufacturer) and Address

S.1.1 Manufacturer (name, manufacturer) :-

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S.1.2 Description of manufacturing process and process controls:-

Manufacturing Process	In process / Quality Control
Seed of *Haemophilusinfluenzae* type b strain Eagan, obtained from PHLS, U.K., identified by record of history, source, tests of characterization to show capability of producing type b polysaccharide.	Record of history and characterization
Seed propagation and establishment of master seed lot (freeze dried). Stored at or below –20°C. Passage level – P0	Control of bacterial purity by morphological, biochemical and immunological tests.
Seed propagation and establishment of working seed lot (freeze dried). Stored at or below –20°C. Passage level – P1	
Preparation of pre-cultures from working seed lot for inoculum for fermenter. (20 ml, 250 ml, 5000 ml)	Bacterial purity, identification by microscopic examination of Gram's stained smears (at least 10,000 organisms are inspected), motility test.
Fermenter culture (110 liters), Passage level – P5	• Culture media sterility • pH control • Dissolved oxygen control. • Temperature control • Rotation speed control • Control of bacterial purity By microscopic examination of Gram's stained smears (at least 10,000 organisms are inspected),motility test, inoculation into solid media.
Harvesting and inactivation by adding formalin (0.5%)	Control of bacterial inactivation.
Bacterial cell separation by continuous flow centrifugation	Control of centrifugation speed.
Precipitation of PRP polysaccharide from culture supernatant by addition of 0.2% cetavalone	• pH control • Temperature control
Dissociation of PRP polysaccharide–cetavalone complex	• Control of centrifugation speed. • Temperature control
Purification of PRP polysaccharide by ethanol precipitation, cold phenol extraction	• Control of centrifugation speed. • Temperature control
Purified polysaccharide lot (Store at or below –20°C)	• Water • Protein • Nucleic acid • Phosphorus • Molecular size • Identification • Bacterial Endotoxins • Ribose • pH • Sterility • Residual reagent ➢ Free Formaldehyde ➢ Cetrimide

Preparation of processed polysaccharide and bulk conjugate lot

Manufacturing Process	In process / Quality Control
Purified polysaccharide lot - Activation with cynogen bromide - Linking with adipic acid dihydrazide (ADH)	• pH Control • Temperature Control
Processed polysaccharide (PRP-AH) - Add tetanus toxoid - Add EDAC - HCl	• pH Control • Temperature Control • Adipic acid dihydrazide content • Molecular size

Bulk conjugate (PRP-AH-TT)
- Add processed polysaccharide
- Add tetanus toxoid
- Add EDAC – HCl
 	
Bulk conjugate lot
(stored at or below -20^{o}C)
	• PRP (Polysaccharide content)
	• Protein
	• PRP to protein ratio
	• Molecular size
	• Free PRP
	• Free carrier protein
	• Test for blood group substance
	• Residual reagents (Unreacted functional groups)
	  • Residual EDAC content
	  • Residual bromide content
	  • Residual cyanide content
	• Sterility

S.1.3 Control of materials:-
(Refer to Point No.S.1.2)

S.1.4 Control of critical steps and intermediates:-
(Refer to Point No.S.1.2)

S.2 Characterization (name, manufacturer)

S.2.1 Elucidation of structure and other characteristics :-

The production of Haemophilus type b conjugate vaccine is based on a seed lot system. The master seed lot used is identified by a record of its history, the source from which it was obtained, and by its biochemical and serological characteristics/ cultures derived from the working seed lot shall have the same characteristics as cultures of the strain from which the master seed lot was derived.
The cultures have following characteristics:-

- Gram negative smear typical of Haemophilus influenza type b.
- Non motile organism.
- Pure dew drop colonies 1-2 mm diameter of glistening mucoid quality.

S.2.1.1Physicochemical Characterization:-

The purified polysaccharide lot of *Haemophilus influenzae* type b is characterized as per the guidelines of Indian Pharmacopoeia.
Analytical testing performed to characterize the *Haemophilus influenzae* type b are follows :-

- Water
- Protein
- Nucleic acid
- Phosphorus
- Molecular size
- Identification
- Bacterial Endotoxins
- Ribose
- pH
- Sterility
- Residual reagent
  - Free Formaldehyde
  - Cetrimide

The Bulk conjugate of *Haemophilus* type b conjugate vaccine is characterized as per the guidelines of Indian Pharmacopoeia.

Analytical testing performed to characterize the *Haemophilus* type b conjugate vaccine are follows :-

- PRP (Polysaccharide content)
- Protein
- PRP to protein ratio
- Molecular size
- Free PRP
- Free carrier protein
- Test for blood group substance
- Unreacted functional groups
  - Residual EDAC content
  - Residual bromide content
  - Residual cyanide content
- Sterility

S.2.1.2 Biological Characterization:-

Each purified polysaccharide lot is tested for identity by rocket immune electrophoresis.

S.2.2 Impurities:-

The impurities such as protein, nucleic acid and bacterial endotoxins were removed during the purification process of the Purified Polysaccharide lot.

S.3 Control of drug substance

S.3.1 Purified polysaccharide lot Specification:-

S. No.	Quality Control Test	Specifications
1	Water	The loss on drying or water content is determined by thermogravimetry method and is used to calculate the results of the chemical tests of purified polysaccharide with reference to the dried substance.
2	Protein	Each lot of purified polysaccharide shall contain not more than 1% protein calculated with reference to the dried substance.
3	Nucleic acid	Each lot of purified polysaccharide shall contain not more than 1% calculated with reference to the dried substance by spectroscopy.
4	Phosphorus	The phosphorus content shall be between 6.8% to 9% as calculated with reference to the dried substance
5	Molecular size	The PRP is identified by immunochemical method.
6	Identification	The identity of the PRP polysaccharide shall be verified by serological method.
7	Bacterial Endotoxins	Not more than 25 I.U. of endotoxins per microgram of PRP.
8	Ribose	Ribose content in purified polysaccharide lot shall be not less than 32% of the dry weight of polysaccharide.
9	pH	The pH value of purified polysaccharide lot of Haemophilus type b conjugate vaccine shall be 7 ± 0.5.
10	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
11	**Residual reagents**	
	Cetrimide	Yellow precipitate formed in standard solution and there should be no precipitation in test sample.
	Free Formaldehyde	0.2 g/l is the maximum limit for free formaldehyde in purified polysaccharide lot of Haemophilus type b conjugate vaccine. The test sample should not be more intense in color than reference solution.

Processed Polysaccharide (PRP - ADH) :-

S. No.	Quality Control Test	Specifications
1.	Adipic acid dihydrazide content	The adipic acid dihydrazide content in processed polysaccharide shall be between 2 ± 0.6%.
2.	Molecular size	The distribution constant (K_D) of processed polysaccharide lot at the main peak of the elution curve shall be between 0.4-0.6.

Bulk Conjugate (PRP-AH-TT):-

S. No.	Quality Control Test	Specifications
1	PRP (Polysaccharide content)	The PRP content is determined by assay of phosphorus as per Indian Pharmacopoeia.
2	Protein	The protein content is determined by chemicals method as per Indian Pharmacopoeia.
3	PRP to protein ratio	The PRP (polysaccharide) to protein ratio of bulk conjugate shall be between 0.3-0.55.
4	Molecular size	Molecular size distribution is determined by size-exclusion chromatographic. The distribution constant (K_D) of bulk conjugate at the main peak of the elution curve shall be <0.2.
5	Free PRP	Unbound PRP (polysaccharide) content in bulk conjugate shall be less than 20%.
6	Free Carrier Protein	Bulk conjugate shall have Free Carrier Protein (unbound protein) content less than 1%.
7	Test for blood-group substances	If an immuno-precipitation band is present in test sample then the test sample fails the test for blood group substances.
8	**Residual reagents**	
	Residual EDAC (ethyldimethyle amminopropyl carbodimide) content	The EDAC content in bulk conjugate shall be less than 25 µmol per ml.
	Residual bromide content	The bromide content in bulk conjugate shall be less than 0.2 µg/ml.
	Residual cyanide content	The Cyanide content in bulk conjugate shall be less than 1 µg/ml.
9	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.

S.3.2 Stability (name, manufacturer) :-

Stability study at real time (at or below -20°C) and accelerated condition (2-8°C) was carried out on three lots of bulk conjugate lot (bulk) of Haemophilus type b conjugate vaccine I.P.
The conditions of study and number of batches considered are satisfactory.
From the result of stability study it was concluded that the drug substance was found to be stable in real time (at or below -20°C) and accelerated condition (2- 8°C). Hence, shelf life of 5 years was assigned for the product under recommended storage conditions (at or below -20°C).

P.1 Manufacturer (Name, dosage form)

P.1.1 Manufacturer (Name, dosage form) :-

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P.1.2 Batch formula:-

The formulated vaccine Peda Hib™ is in freeze dried form and batch formula is given below :-

S.No.	Ingredients	Quantity per single dose (0.5 ml)
1.	Bulk conjugate (polysaccharide of *Haemophilus* type b conjugated to Tetanus Toxoid protein)	10 µg
2.	Lactose I.P.	2 mg
3.	Sucrose I.P.	42.5 mg
4.	Thiomersal I.P. (Preservative)	0.05 mg

P.1.3 Description of manufacturing process and process controls flow diagram:-

Manufacturing Process	Controls
Bulk conjugate, stored at or below -20°C.	
Preparation of final bulk by aseptic dilution with sterile diluent, so as to contain 23 microgram of PRP per ml.	• pH control • Identification • Sterility
Containerization, freeze drying, sealing, visual inspection of final containers, labeling, packing, storage (2-8°C)	• Volume control • Temperature control • Humidity control • Control of freeze drying process
Final lot of *Haemophilus* type b conjugate vaccine I.P.	• Sterility • Abnormal Toxicity Test • Pyrogens • pH • Antimicrobial Preservative • Water • *Haemophilus* type b Polysaccharide or PRP Content • Free PRP Content

P.1.4 Controls of critical steps and intermediates:-

Refer point No.P.1.3

P.2 Control of excipients

P.2.1 Excipients of Human or Animal Origin:-

There is no use of excipient of human or animal origin for the manufacture of Haemophilus type b conjugate vaccine.

P.3 Control of drug product

P.3.1 Specification(s):-

Final Bulk

S. No.	Quality Control Test	Specifications
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
2.	Identification	Purified monospecific polysaccharide shall be shown to be serologically identical & specific.

Final Lot

S. No.	Quality Control Test	Specifications as per Indian Pharmacopeia
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
2.	Abnormal Toxicity Test	The test vaccine passes the test if none of the animal dies or shows signs of ill health in 7 days following the injection. If more than one animal die, the preparation fails the test. If one of the animals dies or show signs of ill health, repeat the test. The test sample passes the test if none of the animals in the second test dies or shows any signs of ill health in the time interval specified.
3.	Pyrogens	If the sum of difference between maximum and initial temperature of three rabbits is less than 1.4°C and if response of individual rabbits is less than 0.6°C, the preparation being examined passes the test.
4.	pH	The pH of final lot of reconstituted vaccine shall be 7 ± 0.5.
5.	Antimicrobial Preservative	Thimerosal content shall be between 0.0085% - 0.0115% per dose of 0.5 ml.
6.	Water	The average residual moisture content of freeze dried final lot vaccine shall not be greater than 3%.
7.	*Haemophilus* type b Polysaccharide or PRP Content	The polysaccharide content of final lot shall be 10 mcg ± 20% per dose.
8.	Free PRP Content	Free PRP (unbound) content in final lot of Haemophilus type b conjugate vaccine (I.P.) shall not be greater than 20 %.

P.3.2 Container closure system:-

Materials used for the final packing of vaccine are as follows:

- **Glass Vials :-**

  2 ml and 5 ml, 13 mm USP type 1 clear tubular glass vial for single and multi-dose.

- **Rubber closures :-**

13 mm Grey Butyl Slotted Rubber Stopper (Sterile ready for use).

- **Aluminium Seals :-**

13 mm flip off PK-1 aluminium seals.

Materials used for the final packing of vaccine diluent are as follows:

- **Glass vial :-**

  2 ml and 5 ml, 13 mm USP type 1 clear tubular glass vial for single and multi dose diluent.

- **Rubber Closures: -**

13 mm Grey butyl, 'Bioclean RFU' Rubber stopper.

- **Aluminium Seals :-**

13 mm flip off white (WE1) aluminium seals.

P.4 Stability

P.4.1 Stability Summary and Conclusion:-

Stability studies real time (2-8^{o}C) and at accelerated condition (20-25^{o}C and 30-35^{o}C) have been conducted on three consecutive lots Haemophilus type b conjugate vaccine. The test results prove good stability of the product. Test specifications for release of final lot were met after storage at recommended storage condition (2-8^{o}C) for at least 48 months. Based on the results of stability studies shelf life of 36 months was assigned for final lot of vaccine at recommended storage condition of +2 to +8^{o}C.

P.4.2 Post approval stability protocol and stability commitment (name, dosage form) :-

Every year one batch of Peda Hib^{TM} is subjected to real time stability study as per the approved protocol.

A. Appendices :- Module 3.2.A

A.1 Details of equipment and facilities for production of drug product

For Layout of the facility used for manufacturing of Peda Hib^{TM} and list of equipments refer to Module 3 Point No. 3.2.A.

A.2 Safety evaluation of adventitious agents

For non-viral adventitious agents :-

The routine manufacturing control of adventitious agents, such as bacteria, mycoplasma and fungi, typically using well-established analytical procedure like sterility test.

Test for sterility is applied to pharmacopoeial articles that are required according to the pharmacopoeia to be sterile.

The test is designed to reveal the presence of micro-organisms in the sample used in the test; interpretation of the results of testing is based on the assumption that all units of an article or the entire bulk product or the contents of every container of the filled product in a lot or batch, had they been tested, would also have given the same results. Since all the units or the bulk or all the containers cannot be tested a sufficient number of samples of units or containers should be examined to give a suitable degree of confidence in the results of the tests.

Media used for the tests should comply with the growth promotion test. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria, soyabean-casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

For viral adventitious agents:-

Haemophilus type b conjugate vaccine is a polysaccharide vaccine there is not found viral adventitious agents.

Materials of Biological Origin:-

There is no material of biological origin used in manufacturing of this product.