Product Permission Document (PPD) of Haemophilus type b conjugate vaccine (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

20F/837/9286 dated 17/07/2004

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

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

Intramuscular

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) :-

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

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

Manufacturing Process	**In process / Quality Control**
Seed of *Haemophilus influenzae* 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°C)
	• 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.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 Haemophilustype b conjugate vaccineis 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.2.1.1 Physicochemical Characterization:-

The purified polysaccharide lot of The Bulk conjugate of *Haemophilus* type b conjugate vaccine is characterized as per the guidelines of W.H.O Technical Report Series No. 897, 2000.

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

- Moisture Content
- Protein
- Nucleic acid
- Phosphorus
- Molecular size
- Identity test
- Bacterial Endotoxins
- Ribose content
- pH
- Sterility
- Residual reagent
- Free Formaldehyde
- Cetrimide

The Bulk conjugate of *Haemophilus* type b conjugate vaccine is characterized as per the guidelines of W.H.O Technical Report Series No. 897, 2000.

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 Specifications of drug substance :-

1. Purified polysaccharide lot:-

S. No.	Quality Control Test	Specifications as per W.H.O. T.R.S. No. 897, 2000
1.	Moisture content	To calculate dry weight
2.	Protein impurity	Protein content shall be less than 1% (10 mg per gram of polysaccharide) by weight of dry polysaccharide as determined by the method of Lowry et. al., using bovine plasma albumin as standard.
3.	Nucleic acid impurity	Each lot of purified polysaccharide shall contain less than 1% (less than 10 mg per gram of polysaccharide) by weight of nucleic acid on the assumption that the absorbance of 10 g/liter nucleic acid solution contained in a cell 1 cm wide at 260 nm is 200.
4.	Phosphorus content	The phosphorus content shall be between 6.8% and 9% of the dry weight of polysaccharide, as determined by the method of Chen et. al. using KH_2PO4 as standard.
5.	Ribose content	Ribose content shall be not less than 32% of the dry weight as estimated by the Bial reaction for pentose, using D-ribose as standard.
6.	Molecular size distribution	The distribution constant (K_D) of polysaccharide at the main peak of the elution curve shall be > 0.3.
7.	Identity test	The identity of the PRP polysaccharide shall be verified by serological method.
8.	Pyrogenicity test	Purified polysaccharide shall pass a pyrogen test (I.P.) in rabbits at 1.0 µg of polysaccharide per Kg of rabbit weight
9.	pH test	The pH value of purified polysaccharide lot of H*aemophilus* type b conjugate vaccine shall be 7 ± 0.5.
10.	Cetrimide	Yellow precipitate formed in standard solution and there should be no precipitation in test sample.
11.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
12.	Free Formaldehyde	0.2 g/l is the maximum limit for free formaldehyde in purified polysaccharide lot of H*aemophilus* type b conjugate vaccine. The test sample should not more intense in color than reference solution.

2. Processed Polysaccharide (PRP - ADH) :-

S. No.	Quality Control Test	Specifications as per W.H.O. T.R.S. No. 897, 2000
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 at the main peak of the elution curve shall be between 0.4-0.6.

3. Bulk Conjugate (PRP-AH-TT):-

S. No.	Quality Control Test	Specifications as per W.H.O. T.R.S. No. 897, 2000
1.	PRP to protein ratio	The PRP to protein ration in bulk conjugate shall be between 0.3-0.6.
2.	Molecular size	The distribution constant (K_D) of bulk conjugate at the main peak of the elution curve shall be between 0.0-0.15.
3.	Free PRP content	Unbound polysaccharide content in bulk conjugate shall be less than 20%.
4.	Blood group substances	If an immuno-precipitation band is present in test sample then the test sample fails the test for blood group substances.
5.	Residual EDAC content	The conjugate purification procedures shall remove the reagents used in conjugation.
6.	Residual Bromide content	The conjugate purification procedures shall remove the reagents used in conjugation.
7.	Residual Cyanide content	The conjugate purification procedures shall remove the reagents used in conjugation.
8.	Sterility	No evidence of microbial growth is observed in any of the inoculated bottles then the preparation being examined 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. 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) :-

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

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 20 microgram of PRP per ml.	• pH control • 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	• Identity • Sterility • Concentration of polysaccharide (PRP Content) • Residual Moisture • Preservative content • Innocuity test • Pyrogen content • Potency Test • pH

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):-

4. Final Bulk :

S. No.	Quality Control Test	Specifications as per W.H.O. T.R.S. No. 897, 2000
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.

5. Final Lot :

S. No.	Quality Control Test	Specifications as per W.H.O. T.R.S. No. 897, 2000
1.	Identity	Purified monospecific polysaccharide shall be shown to be serologically identical & specific
2.	Sterility	Satisfactory (Bacterial & Mycotic Sterility according to the requirements given in W.H.O. T.R.S).
3.	Concentration of polysaccharide (PRP Content)	The polysaccharide content of final lot shall be 10 mcg $\pm$ 20% per dose.
4.	Residual Moisture	The average residual moisture shall be not greater than 2.5% and no vial should be found to have a residual moisture content of 3% or greater.
5.	Preservative content	Thiomersal content shall be between 0.0085%-0.0115%.
6.	Innocuity test	The animals shall be observed for 7 days and the injection shall cause neither significant symptoms nor death during this period (their weight at the end of the test period is not less than that at the time of injection).
7.	Pyrogen content	*Haemophilus* type b conjugate vaccine shall pass a Pyrogen test in rabbits at 0.1 µg of polysaccharide per Kg of Rabbit weight.
8.	Potency Test	Not less than 50% of the vaccinated mice show seroconversion, i.e. they have a titre not less than 4 times that of the pooled control serum.
9.	pH	The p2H shall be between 7.0 $\pm$ 0.5 when reconstituted with the appropriate diluent.

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.