Product Permission Document (PPD) of Typhoid Vi Conjugate Vaccine I.P. (Brand Name – Peda Typh™)

1. Introduction :

Typhoid fever is a generalized acute systemic infection caused by *Salmonella typhi*. It remains a common and serious disease with a disease burden of more than 100 to 1000 per million population annually living in Latin America and some South-East Asian countries. Despite the availability of several antimicrobial agents for its treatment the emergence of antibiotic resistant strains has posed a significant challenge in the treatment of typhoid and so it still continues to remain an important cause for morbidity. Therefore strategies to prevent the disease would include effective sewage treatment, safe potable drinking water and vaccination against the disease.

The vaccines for typhoid currently available in India are the oral attenuated Ty21a vaccine, injectable Vi polysaccharide vaccine and Vi-TT conjugate polysaccharide vaccine.

The Vi-polysaccharide vaccine is said to have some limitations – it is suggested that the immune response is a T cell independent phenomenon and the antibody response is not boosted by additional doses. In order to improve the efficacy of the Vi-polysaccharide vaccine and to protect the susceptible children a new technology for conjugation with a suitable protein was developed by Szu *et al,* 1987. A novel technology in which Vi-polysaccharide vaccine was conjugated using tetanus toxoid has been shown to confer high level of immunogenicity and a T cell dependent immune response.

The Vi-TT vaccine has been manufactured by using adipic acid dihydrazide (ADH) as linker and covalently conjugated to Vi polysaccharide by carbodimide-mediated coupling. Regulatory authorities in India have validated the process of manufacturing and batch consistency has also been established.

A regulatory (pre-licensure) study done across India has demonstrated its immunogenicity and safety in human volunteers. A multicentric regulatory trial of this vaccine was conducted on 169 volunteers aged weeks and above demonstrated that all vaccinated subjects showed a ≥ 4 fold rise of pre-vaccination antibody titres (IgG Vi-antibody). The post vaccination GMT ranged between 62.86-78.66 ELISA units compared to 2.13 to 2.73 pre-vaccination GMT ELISA unit value. Several pre and post licensure clinical trials conducted on Peda Typh™ establish its safety, immunogenicity and efficacy. Immunogenicity and efficacy of the vaccine have been found to be 100%.

1.1. Submission file :-

The product is licensed on Form 28-D, License No. 05/LVP/S&V/2004 permission issued on 1st July, 2008 vide letter no. XXF/837/6820.

1.2. NDS Approval date and control :-

F. No. XXF/837/6820 dated 1st July, 2008 and Drug/837/3285 dated 19/05/2017.

1.3. PPD –Biological revision date and control :-

PPD Biological Revision 04, dated 15/03/2017.

1.4. Proprietary Name:-

Peda Typh™

1.5. Non Proprietary name or common name of drug substance:-

Bulk Conjugate of Typhoid Vi 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/injectables

1.9. Dosage form(s) :-

Peda Typh™ is a clear to slightly turbid solution.

1.10. Strength(s):-

One dose (0.5 ml) contains :-
5 µg of Vi polysaccharide of *Salmonella typhi* (Strain Ty2) conjugated to 5 µg Tetanus toxoid protein in isotonic saline 0.5 ml.

1.11. Route of Administration:-

Intramuscularly

1.12. Maximum Daily Dose :-

Not Applicable

S. Drug substance, name & manufacturer:-

S.1 Manufacturer (name, manufacturer) and address:-

S.1.1 Manufacturer(s) (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 & process control:-

PRODUCTION FLOW DIAGRAM OF MANUFACTURING PROCESS AND IN PROCESS/QUALITY CONTROL TESTS FOR TYPHOID VI CONJUGATE VACCINE I.P.

1. FLOW DIAGRAM FOR MANUFACTURE OF PURIFIED VI POLYSACCHARIDE LOT :

Manufacturing Process	Controls
Strain of *Salmonella typhi Ty2* capable of producing the Vi 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	The cultures have following characteristics: (1) stained smears made from a culture shall be typical of *S. typhi*: (2) the cultures shall utilize glucose without production of gas; (3) the colonies on agar shall be Oxidase-negative; (4) a suspension of a culture shall be agglutinated specifically with an appropriate anti-Vi antiserum or colonies shall form haloes on an antiserum-containing agar plate.
Seed propagation and establishment of working seed lot (freeze dried). Stored at or below -20°C. Passage level – P1	
Preparation of precultures 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 formaldehyde (0.5%)	Control of bacterial inactivation.
Bacterial cell separation by continuous flow centrifugation	Control of centrifugation speed.

Precipitation of Vi polysaccharide from culture supernatant by addition of 0.2% cetavalone (cetrimonium bromide)	•pH control •Temperature control
Dissociation of Vi polysaccharide–cetavalone complex	•Control of centrifugation speed. •Temperature control
Purification of Vi polysaccharide by ethanol precipitation, cold phenol extraction	•Control of centrifugation speed. •Temperature control
Purified Vi polysaccharide lot (Store at or below –20^{o}C)	•Moisture content •Protein content •Nucleic acids content •O-acetyl groups •Molecular size •Polysaccharide content •Identification and serological specificity •Bacterial Endotoxins •pH •Sterility •Residual reagents (Free formaldehyde and Cetrimide)

2. PRODUCTION FLOW DIAGRAM OF PROCESSED PROTEIN AND BULK CONJUGATE LOT:

Purified and concentrated tetanus toxoid bulk - Linking with adipic acid dihydrazide (ADH) in the presence of EDAC (1-ethyl-3-(3-dimethlyaminopropyl) carbodimide) – HCl	• pH Control • Temperature Control • Time control • Reagent concentration
Processed tetanus toxoidTT -ADH	•Hydrazide content
Processed tetanus toxoid TT -ADH - Add purified Vi polysaccharide - Add EDAC – HCl - Purification of bulk conjugate by centrifugation, ultra filtration	• pH Control • Temperature Control • Time control • Reagent concentration
Bulk conjugate Vi -ADH-TT	•Identification •Vi Polysaccharide content • Protein Content •Vi Polysaccharide to protein ratio • Molecular size Distribution •Free Vi (Unbound) polysaccharide •Free Carrier Protein • Residual reagents(EDAC content, residual formaldehyde) • Sterility • Specific toxicity • pH •

3. DESCRIPTION OF MANUFACTURING PROCESS & PROCESS CONTROL OF FINAL BULK VACCINE AND FINAL LOT:

Manufacturing Process	Controls
Bulk conjugate, stored at or below -20°C.	
Preparation of final bulk by aseptic dilution with isotonic saline, so as to contain 5 microgram of Vi polysaccharide per 0.5 ml.	• pH •Osmolality • Sterility
Containerization, sealing, visual inspection of final containers, labeling, packing, storage (2-8°C)	• Volume control • Temperature control • Humidity control
Final lot of Typhoid Vi Conjugate Vaccine I.P.	• Identification • pH • Osmolality • Free Formaldehyde • Free Vi Polysaccharide • Sterility • Pyrogens • Abnormal toxicity test • Assay (Vi Polysaccharide Content) • Potency test • Antimicrobial preservative

S.1.3. Control of materials:-

As in Point No. S.1.2

S.1.4 Controls of critical steps and intermediate:-

As in Point No. S.1.2

S.2. Characterization of drug substance

S.2.1. Elucidation of structure and other characteristics:-

S.2.1.1 Physicochemical Characterization :-

The Bulk conjugate lot of Typhoid Vi Conjugate Vaccine I.P. is characterized as per the specifications of Indian Pharmacopoeia.

Analytical testing performed to characterize the bulk conjugate lot of Typhoid Vi Conjugate Vaccine I.P are follows:-

• Identification
• Vi Polysaccharide content
• Protein Content
• Vi Polysaccharide to protein ratio
• Molecular size Distribution

• Free Vi (Unbound) polysaccharide
• Free Carrier Protein
• Residual reagents (EDAC content, residual formaldehyde)
• Sterility
• Specific toxicity
.pH

S.2.1.2 Biological Characterization :-

Each Bulk Conjugate lot is tested for identity by rocket immune electrophoresis and sterility by direct inoculation method

S.2.2. Impurities:-

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

S.3. <u>Control of drug substance</u>

S.3.1. <u>Specification:-</u>

1. For purified Vi polysaccharide lot :

S. No.	**Quality Control Test**	**Specifications as per Indian Pharmacopoeia 2014,Addendum 2016**
1.	Moisture content	The loss on drying or moisture 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 purified Vi polysaccharide lot shall contain less than 10 mg protein per gram of polysaccharide calculated with reference to the dried substance.
3.	Nucleic acids	Each purified Vi polysaccharide lot shall contain less than 20 mg of nucleic acid per gram of polysaccharide, calculated with reference to the dried substances.
4.	O-Acetyl groups	Not less than 2 mmol per gram of polysaccharide, calculated with reference to the dried substance.
5	Molecular size	In molecular size assay, at least 50% of the Vi polysaccharide is found in the pool containing fractions eluted before a K_o = 0.25.
6.	Identification	The identity of the purified Vi polysaccharide lot with an in house standard Vi polysaccharide shall be established by immunochemical method.
7.	Bacterial endotoxins	Not more than 150 I.U. per microgram of polysaccharide.
8.	pH	The pH value of purified Vi polysaccharide lot of Typhoid Polysaccharide Vaccine shall be 7+0.5.
9.	Cetrimide	Yellow precipitate formed in standard solution and there should be no precipitation in test sample.

10.	Free formaldehyde	0.2 g/l is the maximum limit for free formaldehyde in purified Vi Polysaccharide lot of Typhoid Polysaccharide Vaccine. The sample should not be more intense in color than reference solution.
11.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.

2. For Bulk Conjugate :

S. No.	**Quality Control Test**	**Specifications as per Indian Pharmacopoeia 2014, Addendum 2016**
1.	Identification	The identification of the Vi polysaccharide in bulk conjugate lot shall be established by immunochemical method.
2.	Vi Polysaccharide content	Vi polysaccharide content is determined by Hestrin method.
3.	Protein content	Protein content is determined by Indian Pharmacopeia method and Vi polysaccharide to protein ratio in bulk conjugate shall be between 0.7-1.3.
4.	Vi Polysaccharide to protein ratio	Determined the ratio by calculation. TheVi polysaccharide to protein ratio in bulk conjugate shall be between 0.7-1.3
5.	Molecular size distribution	Molecular size distribution is determined by size exclusion chromatography. The distribution constant (K_D) of bulk conjugate at the main peak of the elution curve shall be between 0.0-0.15.
6.	Free Vi Polysaccharide	Free Vi polysaccharide (unbound) content in bulk conjugate shall be less than 30%.
7.	Free carrier protein	Free carrier protein content in bulk conjugate shall be less than 5%.
8.	Residual Reagents	
	8.1 EDAC content	EDAC content in bulk conjugate should be less than 25 micromoles per ml.
	8.2 Residual formaldehyde	0.2 g/l is the maximum limit for residual formaldehyde in bulk conjugate of Typhoid Vi Conjugate Vaccine. The test sample should not be more intense in color than reference solution.
9.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
10.	Specific toxicity	The bulk toxoid shall pass the test if no guinea pig shows symptoms of specific paralysis or any other signs of tetanus within 21 days of infection and if atleast 80% of the animals survive the test period.
11.	pH	The pH value bulk conjugate shall be 6.5 to 7.5.

S.3.2. Stability:-

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 Typhoid Vi 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 bulk conjugate lot under
recommended storage conditions (at or below -20°C).

P. Drug product (name, Dosage form) :-

P.1. Manufacturer (name, Dosage form):-

P.1.1. Manufacturer(s) (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:-

S.No.	Ingredients	Quantity per dose (0.5 ml) or single dose presentation
1	Purified Vi polysaccharide of *Salmonella typhi* (Strain Ty2) conjugated to tetanus toxoid protein	5 µg
2	Isotonic saline	0.5 ml

P.1.3. Description of Manufacturing process & process control

Manufacturing Process	Controls
Bulk conjugate, stored at or below -20°C.	
Preparation of final bulk by aseptic dilution with isotonic saline, so as to contain 5 microgram of Vi polysaccharide per 0.5 ml.	• pH •Osmolality • Sterility
Containerization, sealing, visual inspection of final containers, labeling, packing, storage (2-8°C)	• Volume control • Temperature control • Humidity control

Final lot of Typhoid Vi Conjugate Vaccine I.P.	• Identification • pH • Osmolality • Free Formaldehyde • Free Vi Polysaccharide • Sterility • Pyrogens • Abnormal toxicity test • Assay (Vi Polysaccharide Content) • Potency test • Antimicrobial preservative(Only for multidose)

P.1.4. Control of critical steps & intermediate

As point No. P.1.3

P.2. <u>Control of excipients:-</u>

P.2.1.Excipients of Human or Animal Origin:

There is no use of excipient that is human or animal origin in the manufacturing of Typhoid Vi Conjugate Vaccine I.P.

P.3. <u>Control of Drug Product:-</u>

P.3.1. Specification (s):

3. For Final Bulk :

S. No.	Quality Control Test	Specifications as per Indian Pharmacopoeia 2014, Addendum 2016
1	pH	The pH value of the final bulk of Typhoid Vi Conjugate Vaccine I.P. shall be 6.5 to 7.5
2	Osmolality	The Osmolality shall be 250 mOsmol/kg to 350 mOsmol/kg.
3	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.

4. For Final lot :

S. No.	Quality Control Test	Specifications as per Indian Pharmacopoeia 2014, Addendum 2016
1.	Identification	The identity of the Vi polysaccharide in final lot of Typhoid Vi Conjugate Vaccine I.P. and in-house standard Vi polysaccharide shall be established by immuno-precipitation.
2.	pH	The pH value of the final lot of Typhoid Vi Conjugate Vaccine I.P. shall be 6.5 to 7.5.
3.	Osmolality	The Osmolality of the vaccine shall be between 250 mOsmol/kg to 350 mOsmol/kg.
4.	Free Formaldehyde	0.2 g/l is the maximum limit for free formaldehyde in Typhoid Vi Conjugate Vaccine I.P. The vaccine should not be more intense in

		colour than reference solution.
5.	Free Vi Polysaccharide	Free Vi polysaccharide (unbound) content in final lot of Typhoid Vi Conjugate Vaccine I.P. shall be less than 30%.
6.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
7.	Pyrogens	If the sum of difference between maximum and initial temperature of three rabbits is less than 1.4^{o}C and if response of individual rabbit is less than 0.6^{o}C, the preparation being examined passes the test.
8.	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 dies, the preparation fails the test. If one of the animals die or show signs of the 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. Animal should show no signs of illness and show no weight loss for a period of seven days.
9.	Assay (Vi Polysaccharide Content)	The estimated amount of polysaccharide per dose is 80% (4 µg/dose) to 120% (6 µg/dose) of the content stated on the label (5 µg/dose).
10.	Potency Test	Not less than 50% of the vaccinated mice show sero conversion i.e. they have a titer not less than 4 times that of the pooled control serum.
11.	Antimicrobial preservative	The content is not less than 85.0 percent and not more than 115.0 percent of the content stated on the label.

P.3.2. <u>Container Closure System (name, dosage form):</u>

Various material used for the final packing of vaccine are as follows:

i) <u>Glass Vial:-</u>
2 ml (for single dose) and 5 ml (for multi dose), 13 mm clear tubular USP type 1 glass vial.

- <u>Rubber closures:-</u>
  13 mm Grey Butyl Rubber Stopper (Sterile ready for use).

- <u>Aluminum Seals:-</u>
  13 mm flip off BE-37 aluminum seals.

ii) Prefilled syringe

USP Type-1 prefilled syringe with fixed needle.

P.4. Stability:-

P.4.1. Stability Summary and Conclusion (name, dosage form) :

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 Typhoid Vi Conjugate Vaccine I.P. 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°C) for atleast 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°C.

P.4.2. Post-approval Protocol and Stability Commitment:

Every year one batch of Peda Typh™ 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 Lay out of the facility used for manufacturing of Peda Typh™ and list of equipments refer to Module 3 Point No. 3.2.A.

A.2. Adventitious Agents Safety evaluation

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 :-
Typhoid Vi Conjugate Vaccine I.P. is a freeze dried preparation therefore there is not found viral adventitious agents.

Materials of Biological Origin :-
There is no material of biological origin used in manufacturing of this product.