Product Permission Document (PPD) of Rabies Vaccine, Human I.P. (Brand Name – SURE RAB™)

1. Introduction :

Rabies has been recognized in humans and animals for many countries, even before biblical times, as a distressing disease that develops rapidly into an acute encephalomyelitis, often frenzied initially, then subsiding into delirium, coma and death. A prominent feature in human is hydrophobia. As efforts to control stray dog population in most Asian countries have not been successful, rabies due to dog bites will continue to pose a serious health hazard for millions of people living in rabies endemic countries.

Though rabies is 100% fatal disease in humans, it is also 100% prevent able if the state of the art modern prophylactic measures are instituted soon after the exposure.

1.1. Submission file :-

F-No. 12-01/BIOMED/14-BD

1.2. NDS Approval date and control :-

- Drug/837/375 dated 16/01/2015 and countersigned by CLA vide Drug/837/6975 dated 26/07/2016.
- Endorsement of IM/ID route of administration vide Drug/837/3284 dated 19/05/2017

1.3. PPD –Biological revision date and control :-

PPD Biological Rev 01, dated 01/06/2017

1.4. Proprietary Name:-

SURE RAB™

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

Bulk material of Rabies Vaccine, Human 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) :-

Reconstitute the freeze-dried lyophilysate, immediately prior to use with entire content of the vaccine diluent provided with the vaccine, gently agitate until lyophilysate is dissolved completely. Dose for adult and infant/child is same.

1.10 Strength (s) :-

Rabies Vaccine, Human I.P. contains:
Rabies virus Pitman Moore (PM) strain propagated in Vero cell, inactivated by β - Propiolactone, potency ≥ 2.5 IU.
Diluent : Sterile water for injection I.P..... 1 ml

1.11 Route of Administration:-

Intramuscular/Intradermal use.

1.12 Maximum Daily Dose :-

Not Applicable

2.0 New Active Substance (NAS) :

Rabies Vaccine, Human I.P. is produced in all over the world for several decades. There is predefined parameters for the manufacturing of rabies vaccine. All the products used in the production of vaccine is already known. Rabies Vaccine, Human I.P. is produced by Bio-Med (P) Ltd. 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,
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e-Mail :bmvaccine@yahoo.com
Website: www.biomed.co.in

S.1.2 Description of manufacturing process and process controls (name, manufacturer)

MANUFACTURING PROCESS	CONTROL/TESTS

W.H.O. VERO Master cell bank, lot No.10-87, passage No.134, characterized & deposited by W.H.O. and distributed by A.T.C.C., U.S.A.
(Meets WHO requirements for continuous cell lines used for biological production as detailed in W.H.O., T.R.S. No.878, 1998).
 	
Manufacturer's working cell bank (MWCB), passage No. 138.
	(Collaborative studies for control tests done in 10 laboratories by W.H.O.)
	• Sterility tests
	• Adventitious agents
	• Tumorigenicity in newborn rats
	• Reverse transcriptase assay for tests for retroviruses.
	• Identity test
	• Sterility tests for fungi and bacteria and mycoplasma
	• Tests for viral agents using cell cultures
	  • MNA cells
	  • VERO cells
	  • MRC-5 cells
	• Tests for viral agents using animal and eggs
	  • Suckling mice
	  • Adult mice
	  • Guinea-pig
	  • Rabbit
	  • Embryonated chicken egg
	• Validation of cryopreservation by cell viability tests

MANUFACTURING PROCESS	CONTROL/TESTS

Rabies virus Pitman-Moore (PM) strain adapted in cell culture originated from Pasteur's strain of 'fixed' virus.
 	
Rabies virus Pitman-Moore (PM) strain, Primary virus seed lot No. PMPV-PVS-01 dated 16/17/2002, Lyophilized 0.5 ml per vial. Prepared by infecting VERO cells (MWCB lot No.WV1 dated 29/11/02).
	• Sterility tests for bacteria, fungi and mycoplasma
	• Tests for adventitious agents in animals
	  • Suckling mice
	  • Adults mice
	  • Guinea-pigs
	• Test for adventitious agents in cell cultures
	  • VERO cells
	  • MNA cells
	  • MRC-5 cells

Rabies virus Pitman-Moore (PM) strain, Secondary virus seed lot.	• Virus content
	• Identification
	• Sterility tests for bacteria, fungi and mycoplasma
	• Tests for adventitious agents in animals
	  • Suckling mice
	  • Adults mice
	  • Guinea-pig
	• Tests for adventitious agents in cell cultures
	  • VERO cells
	  • MNA cells
	  • MRC-5 cells
	• Virus content
	• Identification

MANUFACTURING PROCESS	CONTROL/TESTS
Manufacturer's working cell bank of VERO cells (Passage No.138)	• Monitoring cell growth • Temperature control
Revival and propagation (Passage No.142)	• Cell counting
Control cells (5%)	• Monitoring cell growth • Temperature control
Cell suspension (95%)	• Test for haemadsorbing viruses

Infect with rabies virus Pitman-Moore (PM) strain, secondary virus seed lot at an input of multiplicity of infection of about 1 LD_{50} per 1000 cells	• Tests for other adventitious agents in cell culture
	  • VERO cells
	  • MNA cells
	  • MRC-5 cells
	• Identity test

Multiple virus harvests	• pH control
Pooling for preparation of single harvest	• Dissolved oxygen control • Temperature control • Monitoring cell growth
Clarification using 0.45 μ filter	• Sterility test • Identification test • Virus concentration

MANUFACTURING PROCESS	CONTROL/TESTS

Virus concentration and purification using continuous flow sucrose gradient ultra-centrifugation	• Temperature control
	• Tests for effective inactivation
	  • Adult mice
	  • Rabies virus amplification test

Inactivation using 1:4000 (v/v) β-propiolactone	• Validation of purification process
Filtration by 0.2 μ membrane porosity, dispensed in bottles, sample take for quality control testing (storage at or below -20°C)	• Rabies virus amplification test for effective inactivation. • Antigen content • Test for adventitious agents • Test for haemadsorbing viruses • Residual DNA content • Identity test for VERO cells • Residual animal serum

S.1.3 Control of materials (name, manufacturer) flow diagram

As discussed in the point No. S.1.2.

S.1.4 Control of critical steps and intermediates (name, manufacturer)

As discussed in the point No. S.1.2.

S.2 Characterization (name, manufacturer)

S.2.1 Elucidation of structure and other characteristics

Rabies Vaccine, Human I.P. is produced in continuous VERO cells line. The use of VERO cell line is based on a control cell seed lot system. Study on VERO cell with respect to sterility, identity, adventitious agents, tumorogenicity, presence of reverse transcriptase showed the safe use of VERO cell for the production of RABIES VACCINE, HUMAN I.P. from the master cell bank working cell bank lot was prepared.

The strain of virus (Pitman Moore) used in the production was well characterized laboratory adapted and attenuated with stable biological characteristics. The Pitman Moore (PM) strain of rabies virus originated from the Pasteur Strain of rabies 'fixed' virus.

From the master seed lot primary and secondary virus seed lot were prepared. Several test was done on primary & secondary virus seed lot of rabies virus including titration of rabies virus in mice, identity, sterility (bacterial & mycotic) mycoplasma, test for adventitious agent.

The purified bulk material is characterized by conducting test(s) test for Rabies virus amplification test for effective inactivation, Antigen content, Adventitious agents, Haemadsorbing viruses, Residual DNA content, Identity test for VERO cells and Residual animal serum.

The control cell culture are characterized by conducting test(s) observation for cytopathic agents, haemadsorbing virus, identity (isoenzyme analysis) and adventitious agents in cell culture.

Biological Characterization :-

Primary and Secondary virus seed lot

The primary virus seed lot and secondary virus seed lot of Rabies virus are characterized for Titration of rabies virus in mice, Identity, Sterility test for bacteria, fungi and mycoplasma, Tests for adventitious agents using cell cultures and Tests for adventitious virus by animal inoculation.

Manufacturer's Working Cell Bank of VERO cells

The manufacturing working cell bank of VERO cells are characterized for Identity test (isoenzyme analysis), Sterility test, Tests for Viral Agents using Cell Cultures, Tests for Viral Agents using Animals and Eggs.

Control Cell Culture

At least 5% of production cell cultures set aside as control cells are observed for cytopathic agents, haemadsorbing agents, identity and for adventitious agents in cell cultures.

Purified Bulk Material

The purified bulk material is characterized by conducting test(s) test for Rabies virus amplification test for effective inactivation, Antigen content, Adventitious agents, Haemadsorbing viruses, Residual DNA content, Identity test for VERO cells and Residual animal serum.

S.2.2 Impurities (name, manufacturer)

Impurities in Rabies Vaccine, Human I.P. can arise from the following

The virus seed lot(s) :-

The Pitman-Moore (PM) strain of rabies virus originated from the Pasteur strain of rabies 'fixed' virus. The strain has been characterized by serological and animal inoculation. The strain produces characteristic paralysis within 5-7 days when inoculated intracerebrally into rabbits and mice. Most modern cell culture vaccines prepared from the strains that originate from the Pasteur strain of rabies fixed virus have been shown to yield safe and immunogenic vaccines. Currently available human diploid cell rabies vaccine and purified Vero cell rabies vaccine are prepared from Pitman-Moore strain. These vaccines have been shown to protect against local field rabies virus infection in India and Worldwide.

As per the recommendation given in WHO TRS 824 "It is important that WHO Collaborating Centers serve as source of vaccine seeds, as reference laboratories for examination of strains and for training in control techniques".

The Pitman-Moore strain of rabies virus was made available to Bio-Med (P) Ltd. by Centers of Disease Control and Prevention (CDC), Atlanta GA 30333. U.S.A. (a WHO Collaborating Center). Training in virus propagation in cell culture, identification, RFFIT and control techniques were also given at the rabies section at CDC Atlanta.

The manufacturer's working cell bank

A quantity of cells of uniform composition desired from the master cell bank at a finite passage level, dispensed in aliquots into individual containers appropriately stored, usually frozen at – 100°C or below, one or more of which would be used for production purposes. All containers are treated identically and once removed from storage, are not returned to the stock.

To ensure the uniform composition of the contents of each container, a single pool of cells for banking should be prepared by combining the cells from all of the culture vessels, if more than one vessel is used.

Cells suspended in preservation medium are aliquoted from the single pool into sterilized containers, which are then sealed and stored under appropriate conditions. The manufacturers working cell bank have been characterized as per the requirement of the Indian Pharmacopoeia.

The culture media containing fetal calf serum

Since the Rabies viruses is highly cell associated the culture media containing the fetal calf serum is decanted and removed before harvesting. Further the virus infected cell layer is washed three times with phosphate buffer saline virtually eliminating the bovine serum albumin. The manufacturing process is validated by estimation of bovine serum albumin concentration in the purified bulk material of Rabies vaccine.

S.3 Control of drug substance

S.3.1 Specification :-

A. VIRUS HARVEST

Name of Test	Specification as per Indian Pharmacopoeia
Test for Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
Test for Mycoplasma	The product passes the test if growth of Mycoplasmas has not occurred in any of the inoculated media. If growth of Mycoplasmas has occurred, the test may be repeated once with twice the amount of inoculum and media; if growth of Mycoplasmas does not occur when the test is repeated, the product passes the test.
Virus concentration	In-house specification of virus titer of single virus harvest has been set at $>10^{7.0}$ /ml.

B. BULK MATERIAL

Name of Test	Specification as per Indian Pharmacopoeia
Inactivation (Test for Rabies virus amplification test for effective inactivation)	None of the mice inoculated shows any sign/symptoms due to rabies virus as confirmed by immunofluorescence assay.
Antigen content in bulk material	Antigen content of bulk material should be greater than 100 IU per ml by antibody binding assay.
Residual host-cell DNA	The content of residual host-cell DNA should not be greater than 10 ng per single human dose.

C. CONTROL CELL CULTURE

Name of Test	Specification as per Indian Pharmacopoeia
Test for extraneous agents	No evidence of extraneous agents is found.
Test for Haemadsorbing virus	No evidence of haemadsorbing agents is found.
Test for Identity (Isoenzyme analysis)	The cell line tested shall be positive for VERO cell line by comparing the corrected migrated distances of the test sample to known values for the species.

S.3.2 Stability summary and conclusions

S.3.2.1 Protocol of stability study, results and conclusions

Purified bulk material of Rabies Vaccine, Human I.P., is stable at real time and temperature i.e.at or below -20^{o}C for 4 years and at accelerated condition i.e. at 2 to 8^{o}C for 3 months.

P. Drug Product (Name, dosage form)

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 (name, dosage form)

Rabies Vaccine, Human I.P. contains:

Ingredients	**Quantity**
Rabies virus Pitman Moore (PM) strain propagated in Vero cell, inactivated by β -Propiolactone, potency	≥ 2.5 IU
Stabilizer: Polygeline, Sucrose, Salts Diluent consists of sterile water for injection.... 1.0 mL Diluent : Sterile water for injection I.P..... 1 ml	

P.1.3 Description of manufacturing process and process controls (name, dosage form) flow diagram

MANUFACTURING PROCESS	**CONTROL / TESTS**
Purified bulk material (stored at or below -20°C) ↓ Aseptic dilution by virus stabilizer for preparation of final bulk ↓ Filling, containerization and lyophilization ↓ Sealing ↓ Visual Inspection ↓	Purified Bulk Material • Antigen content test • Residual DNA content test • Test for residual animal serum • Rabies virus amplification test for effective inactivation Final bulk • Sterility • Test for determination of antigen content

Labeling & Packing (Final Lot) (Store at 2°C to 8°C) The	Final lot • Sterility • Bacterial endotoxins • Test for Abnormal toxicity • Water (Residual moisture) • Accelerated degradation • Bovine Serum Albumin • Residual host-cell DNA • Assay for identification, potency in mice by NIH Test

final bulk of Rabies Vaccine, Human I.P. is prepared by aseptic dilution of the purified bulk material based on the antigen content. The dilution of the purified bulk material for the preparation of final bulk is done by virus stabilizer containing sucrose, polygeline (haemaccel), potassium L-Glutamate, EDTA.

The final lot is prepared by aseptically dispensing the final bulk vaccine, in grade A conditions with grade B background, in 2 ml capacity tubular glass vials which have been final rinsed with water for injection I.P. and sterilized in dry heat sterilizer. During filling the volume of filling, room temperature, humidity, microbial load is periodically monitored.

Vials are half stoppered with butyl stoppers (pre-sterilized by radiation), collected and loaded into freeze drier. The lyophilization cycle have been validated and the moisture content of consecutive six batches of final lot of Rabies Vaccine, Human I.P. meets the specifications of Indian Pharmacopoeia. After lyophilization cycle sterile nitrogen is introduced in the lyophilizer and vials are full stoppered. On removal from the lyophilizer the vials are sealed with aluminium caps. The sealed vials are visually inspected, labeled, packed and stored at 2-8°C.

P.1.4 Controls of critical steps and intermediates (name, dosage form)

Same as in point no. P.1.3

P.2 Control of excipients (Name, dosage form)

P.2.1. Excipients of Human or Animal Origin (name, dosage form):

There is no use of excipient of human or animal origin for the manufacture of SURE RAB™ vaccine.

P.3 Control of drug product (Name, dosage form)

P.3.1 Specification(s) (name, dosage form)

For Final Bulk

S. No.	Test performed	Specifications
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
2.	Test for determination of antigen content	The estimated potency value of antigen content in final bulk shall not be less than 2.5 I.U. per 0.5 ml (filled volume of final bulk is 0.5 ml per vial).

For Final Lot

S. No.	Test performed	Specifications
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
2.	Bacterial Endotoxins	Not more than 25 I.U. per human dose of 1 ml (per vial).
3.	Water (Test for residual moisture)	The average residual moisture content of freeze-dried final lot vaccine shall not be greater than 3%.
4.	Abnormal Toxicity	The test vaccine passes the test if none of 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 die or show signs of ill health, repeat the test. The test sample passes the test it none of the animals in the second test dies or show any signs of ill health in the time interval specified.
5.	Accelerated Degradation	The vaccine complies the test if the estimated potency is not less than 2.5 I.U. per single human dose.
6.	Bovine Serum Albumin	Length of rocket of test sample should be less than the length of Rocket of standard containing 50 ng /ml of BSA. Not more than 50 ng per single human dose.
7.	Residual host cell DNA	The content of residual host –cell DNA should not be greater than 10 ng per single human dose.
8.	Assay for identification, potency in mice by NIH Test	The assay serves to identify the vaccine. The vaccine complies the test if the estimated potency is not less than 2.5 I.U. per single human dose.

P.3.2 Container closure system (name, dosage form)

Materials used for the final packing of Rabies Vaccine, Human I.P. are as follows:

- Glass Vials :-

  USP Type-I clear tubular 2 ml glass vials.

- 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 (Sterile Water for Injection I.P.) are as follows:

- Glass Vials :-

  USP Type-I clear tubular 2 ml glass vials.

- Rubber closures :-

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

- Aluminium Seals :-

  13 mm flip off WE-1 aluminium seals.

P.4 Stability (Name, dosage form)

P.4.1 Protocol of stability study, results and conclusions

Stability studies real time (2-8^{o}C) and at accelerated condition (20-25^{o}C) and (37 + 1^{o}C) have been conducted on three consecutive lots of Rabies Vaccine, Human I.P. manufactured from three different bulk lots. 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^{o}C to +8^{o}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^{o}C to +8^{o}C.

Stability studies of three consecutive lots of vaccine diluent (sterile water for injection I.P.) for Rabies Vaccine, Human I.P. when kept at room temperature for 42 months comply with the release specifications of final lot for the tests performed.
Freeze dried vaccine after reconstitution with vaccine diluent was tested for stability at 20-25^{o}C for upto 120 minutes. The test results prove good stability of the reconstituted vaccine at the conditions tested. The reconstituted vaccine at 20-25^{o}C for 120 minutes met the final lot specification as per I.P.

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

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

A. Appendices :-Refer to 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 SURE RAB^{TM} and list of equipments refer to Module 3 Point No. 3.2.A.

A.2 Safety evaluation of adventitious agents

Rabies Virus Seed Lot(s)

The master seed lot and working seed lot of Rabies virus are characterized for absence of adventitious agents using cell culture, animal inoculation, mycoplasma, bacteria and fungi.

VERO cells

The manufacturing working cell bank of VERO cells are characterized for absence of viral agents using cell culture, animal inoculation, egg inoculation, mycoplasma, bacteria and fungi.

Control Cell Culture

At least 5% of production cell cultures set aside as control cells are observed for identity test, haemadsorbing agents and for adventitious agents in cell cultures.

Virus harvest

Virus harvest of Rabies Vaccine, Human I.P. is characterized for absence of adventitious agents by sterility test for bacteria and fungi.

Purified Bulk Material

Purified bulk material of Rabies Vaccine, Human I.P. is characterized for absence of adventitious agents by sterility test for bacteria and fungi.

Final Bulk

Final Bulk of Rabies Vaccine, Human I.P. is characterized for absence of adventitious agents by sterility test for bacteria and fungi.

Final Lot

Final lot Rabies Vaccine, Human I.P. is characterized for absence of adventitious agents by sterility test for bacteria and fungi.