Product Permission Document (PPD) of Varicella Vaccine, Live I.P.
Brand Name: Bio Pox™

1. Introduction :

Membership of a virus to the family of herpesviruses is based on the architecture of its virion. The size of virions varies between 120 and 300 nm. They are described as round or polygonal particles with a clear visible central dot.

All herpesviruses contain one copy of a linear and double-stranded DNA coiled upon a protein axis. This formation of linear DNA and proteins is called core. During transition of the DNA from the capsid into the nucleus of an infected cell, the DNA changes from a linear into a circular state.

The VZV genome is 125 kb in size and contains at least 69 different open reading frames (ORF). The genome of the VZV is the smallest of all known human herpesviruses, ranging between 120 and 230 kb. Likewise, the GC content varies from 31 to 75%.

The core is surrounded by an icosahedric capsid (also known as nucleocapsid) with 100-110 nm in diameter. This structure is composed of exactly 162 proteins, the capsomers. It is not possible ti distinguish the VZV capsid from those of other herpesviruses morphologically. All capsomeres occur in a 5:3:2 axial symmetry in which pentameric proteins form the vertices of an 80- to 120 –nm icosahedrons. Hexameric elements comprise its facets. Products of ORFs 20, 23, 33, 33.5, 40 and 41 are concerned in morphogenesis of the nucleocapsid.

An amorphous structure, the tegument, is located between the capsid and the external envelope. The product of ORF10 is located in the VZV tegument.

In addition it includes immediate-early proteins encoded by ORFs 4.62 and 63.

The outer covering or envelope of all herpesviruses is reported to have a typical trilaminar appearance. This particle captures membranous elements of a lot of different cell organelles from the host cell that are trimmed with lipids. During the transport of virions along different membrous networks like nuclear membranes, Golgi apparatus, rough endoplasmic reticulum, cytoplasmic vesicles and elements of the cell surface, modifications of the envelope are performed. At the end, the enveloped particles have a pleomorphic to spherical shape with a diameter of 180-200 nm. Spikes interspersing the envelope are made up of glycoproteins. The length of the spikes is approximately 8 nm.

Varicella Vaccine, Live I.P. is a freeze dried preparation of attenuated OKA strain of Varicella-Zooster virus obtained by the propagation of the virus in MRC-5 human diploid cell culture. Bio Pox™ is indicated for active immunization against Varicella virus (Chicken Pox) of healthy subjects with no history of Varicella infection and susceptible healthy close contacts from the age of 12 months onwards. Bio Pox™ is manufactured as per Indian Pharmacopoeia.

The OKA strain is recommended by Indian Pharmacopoeia, European Pharmacopoeia and WHO.

1.1. Submission file :-

(a) File No. 12 – 32/ BIOMED/11 – BD
(b) File No. 12 – 19/ BIOMED/16 – BD

1.2. NDS Approval date and control :-

Drug/837/5424 dated 27/10/2017

1.3. PPD –Biological revision date and control :-

PPD Biological Rev 01, dated 01/11/2017.

1.4. Proprietary Name:-

BIO POX™

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

Clarified virus harvest (Bulk) of Varicella Vaccine, live I.P.

1.6. Company Name :-

BIO-MED (P) LTD.
C-96, Site No. 1,
Bulandshahr Road Industrial Area,
Ghaziabad - 201009 (U.P.) INDIA
Phone : 0120-4157534, 4159857
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) :-

Freeze dried vaccine on reconstitution with Sterile water for injection I.P. through subcutaneous route.

1.10. Strength (s) :-

Each single dose (0.5 ml) of reconstituted Bio Pox™ vaccine contains:

- $\geq$ 2000 plaque forming units (PFU) of live attenuated Varicella virus (OKA strain) propagated in MRC-5 human diploid cell culture.
- Excipients : KH_2PO_4 - 0.25 mg, K_2HPO_4 - 0.6 mg, L-monosodium glutamate - 0.4 mg, EDTA - 1 mg, Sucrose - 37.5 mg, Human Normal Albumin - 5 mg, Polygeline (Haemaccel®) - 5 mg.

Vaccine Diluent (Sterile water for injection I.P.)

1.11. Route of Administration:-

Subcutaneous

1.12. Maximum Daily Dose :-

Not Applicable.

2.0 New Active Substance (NAS) :

VARICELLA VACCINE, LIVE I.P. is produced in all over the world for several decades. There is predefined parameters for the manufacturing of VARICELLA VACCINE, LIVE I.P. All the products used in the production of vaccine is already known. VARICELLA VACCINE, LIVE 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 and address:-

S.1.1. Manufacturer:-

BIO-MED (P) LTD.
C-96, Site No. 1,
Bulandshahr Road Industrial Area,
Ghaziabad - 201009 (U.P.) INDIA
Phone : 0120-4157534, 4159857
Fax : 0120-4340219
E-mail : bmvaccine@yahoo.com
Website : www.biomed.co.in

S.1.2. Description of manufacturing process & process control:-

1. Preparation and characterization of Manufacturer's Working Cell Bank of MWCB of MRC-5 cell line	
Manufacturing Process	**Control / Tests**
1.1 Cell Seed A quantity of fully characterized cells of human organ stored frozen at -70°C or below in aliquots of uniform composition, one or more of which are used for the production purposes. Cell Line Designation: MRC-5 (Lung fibroblast) ATCC Catalogue No. : CCL-171 Lot No. : 3379608 Ampoule Passage No. : 15 Population Doubling (PDL) : 23 Date Frozen : 11/21/03	• DNA profile (STR Analysis) • Reverse transcriptase assay for tests for retroviruses. • Identity Test

1.2 Manufacturer's working cell bank (MWCB)
 	
A quantity of cells of uniform composition derived from one or more ampoules of the cell seed and stored frozen at -70°C or below in aliquots, one or more which are used for production purposes.
 	
Passage No. : 20, PDL 31.5
 	
Lot No. : WMRC-1, Dated 29/08/2005, 70 cryo vials
 	
Cell Concentration : 2 x 10^6/ml
 	
Volume : 1 ml/cryo vial
	• Identity test by isoenzyme analysis
	• Sterility tests (fungi and bacteria)
	• Test for Mycoplasma
	• Tests for extraneous agents using cell cultures
	  ◦ VERO cell culture
	  ◦ MRC-5 cell culture
	• Tests for extraneous agents using animal and eggs
	  ◦ Suckling mice
	  ◦ Adult mice
	  ◦ Guinea-pig
	  ◦ Rabbit
	  ◦ Embryonated chicken egg
	• Validation of cryopreservation by cell viability tests
	• Test for mycobacteria by culture method
	• Test for retroviruses by reverse transcriptase assay

2. **Preparation of characterization of master seed lot and working seed lot of Varicella virus.**	
Manufacturing Process	**Control / Tests**
2.1 <u>Source and history of Live, attenuated Varicella virus vaccine strain</u> ATCC Catalogue No. : VR-795 Classification : Herpes Virus, Human, Varicella Virus Agent : Varicella Strain : Oka (Live, attenuated Varicella vaccine strain) Original Source : Vesicle, 3 years old male, Japan (Reference : Takahashi, M. et al., Biken J. 18:25-33, 1975) Lot No. : 217504 (Lot 7W) Titer : $10^{3.5}$ $TCID_{50}$/0.2 ml Lot Prepared By : ATCC, USA	Identity : Confirmed by FA using Bio Whittaker MAb for Varicella IgM Cat # 51-367L

Manufacturing Process	**Control / Tests**

2.2 <u>Master Seed Lot</u>
 	
A quantity of virus suspension that has been processed as a single lot to ensure a uniform composition and is fully characterized. The master seed lot is used for the preparation of working seed lots.
 	
Cells used for preparation (homogenous to those to be used for production of the final vaccine) : MRC-5 cells Lot No. WMRC-1 Dated 29/8/2005
 	
Varicella virus (live, attenuated) Master Seed Lot
 	
Storage temperature : below -20°C
 	
Lot No. : OKA-MSL-01
 	
Dated : 08/10/2005
 	
Strain : OKA
	• Identity test
	• Sterility tests (fungi and bacteria)
	• Test for mycoplasma
	
	• Tests for extraneous agents using cell cultures
	  ◦ VERO cells
	  ◦ MRC-5 cells
	
	• Tests for extraneous agents using animal and eggs
	  ◦ Suckling mice
	  ◦ Adult mice
	  ◦ Guinea-pig
	
	• Assay (Virus Concentration)
	
	• Test for mycobacteria

Passage : 34 (ATCC + 2 passages) Virus titer : $10^{4.8}$ PFU/ml Volume : 0.5 ml per vial, lyophilized (Reconstitute with 0.5 ml WFI) Total No. of vials : 950	• Control cell culturesm ➢ Test for observation for cytopathic agents ➢ Test for haemadsorbing viruses ➢ Test for identity ➢ Test for extraneous agents in cell culture

Manufacturing Process	**Control / Tests**

2.3 <u>Working Seed Lot</u>
 	
A quantity of fully characterized virus of uniform composition, derived from a master seed lot (Master Seed Lot No. OKA-MSL-01, dated 08/10/2005). The working seed lot is used for the production of vaccines.
 	
Cells used for preparation (homogenous to those to be used for production of the final vaccine) : MRC-5 cells Lot No. WMRC-1 Dated 29/08/2005
 	
Varicella virus (live, attenuated) Working Seed Lot
 	
Storage temperature : below -20°C
 	
Lot No. : OKA-WSL-01
 	
Dated : 20/10/2005
 	
Passage No. : 35 (ATCC + 3 passages)
 	
Virus titer : $10^{5.2}$ PFU/ml
 	
Volume : 0.5 ml per vial, lyophilized (Reconstitute with 0.5 ml WFI)
 	
Strain : OKA
 	
Total No. of vials : 950
	• Identity test
	• Sterility tests (fungi and bacteria)
	• Test for mycoplasma
	• Tests for extraneous agents using cell cultures
	  • VERO cells
	  • MRC-5 cells
	• Tests for extraneous agents using animal and eggs
	  • Suckling mice
	  • Adult mice
	  • Guinea-pig
	• Assay (Virus Concentration)
	• Test for mycobacteria
	• Neurovirulence test
	
	<u>Control cells</u>
	• Test for observation for cytopathic agents
	• Test for haemadsorbing viruses
	• Identity test (Isoenzyme analysis)
	• Tests for extraneous agents using cell cultures
	  • VERO cells
	  • MRC-5 cells

Manufacturing Process	Control / Tests
3. Propagation and Harvesting MRC-5 cells from one cryo vial of Manufacturer's Working Cell Bank (Lot No. WMRC-1, dated 29/08/2005, Passage No. 20 and PDL 31.5) ↓ Revival and propagation of MRC-5 cells ↓ **Propagation of virus for preparation of inoculum** – infection with cell free working seed lot of Varicella virus (OKA strain) at an established M.O.I. ↓ ≃ 95% cells + Infection by cell associated virus at an established M.O.I. | Control cells ≥ 5% ↓ **Harvesting of virus infected cells.** Culture media is decanted and then cell sheet is washed three times with P.B.S. pH 7.2 ± 0.2. Add virus stabilizer with detachment buffer followed by mechanical shaking for cell harvesting ↓ Sonication for cell lysis at strength 20 KHz for 30 seconds for virus harvesting ↓ Sample pooled virus harvest was taken for quality control testing (storage at or below -60°C) ↓	**Control cells** • Test for observation for cytopathic agents • Haemadsorbing viruses (agents) • Test for Identity (Isoenzyme analysis) • Other extraneous agents tests in cell cultures **Virus harvest** • Sterility • Test for absence of mycoplasmas. • Mycobacteria • Identification • Virus Concentration (Assay) • Extraneous agents.

Manufacturing Process	Control / Tests
Clarification of virus harvest by centrifugation at 300 g ((± 100g) for 5 minutes at 5° ± 3°C ↓ Pooled supernatant dispensed in bottles, sample taken for quality control testing (storage at or below -60°C) ↓ Aseptic dilution by virus stabilizer for preparation of final bulk ↓ Filling, containerization and lyophilization ↓ Sealing ↓ Visual Inspection ↓ Labelling & Packing (Store at 2°C to 8°C)	**Clarified virus harvest** • Sterility • Test for intact cell • Virus Concentration (Assay) **Final bulk** • Sterility **Final lot** • Sterility • Identification • Assay (virus concentration) • Abnormal toxicity • Water • Bovine serum albumin

S.1.3. Control of materials :-

As discussed in the point No. S.1.2.

S.1.4. Control of critical steps & intermediate:-

Cell Seed

The original seed stock of the MRC-5 culture was frozen in 1966 at population doubling level (PDL) 7.

It was frozen in 481 glass ampoules in 54 pools, each containing 7-10 ampoules per pool (Jacobs et al., 1970). MRC-5 cells were found to support the growth of a range of human viruses and were established as a cell type that could be used for vaccine manufacture. The original stock of "PDL 7" cells has been released by NIBSC for vaccine development for a number of decades (Wood and Minor, 1991). MRC-5 cells can be maintained in vitro for approximately 50 population doublings, at which point they becomes senescent and cease to replicate i.e. they are a `finite cell line'.

The main advantage of diploid cell lines in comparison to primary cells is that they can be well characterized and standardized and production can be based on a cell bank system. In addition, unlike the continuous cell lines, they possess a finite life and are not tumorigenic. The cell bank system usually consists of cell banks of defined passage levels.

Bio-Med (P) Ltd. requested American Type Culture Collection for supply of WHO MRC-5 Cells, P15, catalog No. CCL-171.

The MRC-5 cell line was derived from normal lung tissue of a 14-week-old male fetus by J.P. Jacobs in September of 1966.

Chromosome Frequency Distribution 50 Cells : 2n = 46. This is a normal diploid human cell line with 46,XY karyotype. The modal chromosome number was 46, occurring in 70% of cells. The rate of polyploidy was 3.6%. Both X and Y chromosomes were normal.

A quantity of cells of uniform composition desired from the Cell seed at a finite passage level, dispensed in aliquots into individual containers appropriately stored, usually frozen at or below,-60°C one or more of which would be used for production purposes.

Cells suspended in preservation medium are aliquoted from the single pool into sterilized containers, which are then sealed and stored under appropriate conditions.

Manufacturer's working cell bank lot No. :-

Tests employed for the characterization of manufacturers working cell bank lot.

- Identity test
- Sterility test for bacteria, fungi and mycoplasma
- Tests for extraneous using cell cultures
- Tests for extraneous using animals and eggs
- Validation of cryopreservation by cell viability test.
- Test for mycobacteria
- Test for Retro viruses

<u>Varicella virus used for production of Varicella vaccine</u>

The OKA strain of Varicella virus was contributed by the Research Foundation for Microbial Diseases, Osaka University to ATCC where further four passages were made in MRC-5, human diploid cells (ATCC CCL171) to prepare lot 7W of VR-795.

The OKA strain of Varicella virus was made available to Bio-Med (P) Ltd. by ATCC, USA.

Virus seed lots shall be maintained in dried or the frozen form & each lot shall be stored separately.

Master Seed Lot

A quantity of virus that has been processed as a single lot and has a uniform composition is used for the preparation of working seed lot.

Test employed for characterization of master seed lot of Varicella virus OKA strain.

- Tests for mycoplasma
- Tests for mycobacteria
- Tests for extraneous agents using cell culture
- Tests for extraneous agents by animal inoculation
- Tests for sterility
- Tests for assay (virus concentration)
- Identity test

Working seed lot

- Tests for mycoplasma
- Tests for mycobacteria
- Tests for extraneous agents using cell culture
- Tests for extraneous agents by animal inoculation
- Tests for sterility
- Tests for assay (virus concentration)
- Identity test

Manufacturer's working cell bank of MRC-5 cells, passage No. 31 was infected with Varicella virus OKA strain, working seed lot at an input of multiplicity of infection of 2000 PFU per 175 cm^2 culture flask.

Stage/Manufacturing Process		Control test employed
Control cells (5%)	:	• Tests for observation for cytopathic Agents • Tests for haemadsorbing virus • Test for identity (isoenzyme analysis) • Test for extraneous agents in cell cultures

Step		Tests
Sonication for cell lysis	:	• Identification • Virus Concentration(Assay) • Extraneous agents • Sterility • Mycobacteria • Test for absence of Mycoplasmas • Tests for extraneous agents (in neutralized Virus harvest) using cell cultures - VERO cells - MRC-5 cells
Clarification of virus harvest by centrifugation at 300 g (± 100g) for 5 minutes at $5^o \pm 3^oC$	:	• Sterility test • Test for intact cell • Assay (virus concentration)
Aseptic dilution by virus stabilizer for preparation of final bulk	:	• Sterility test
Filling, containerization And lyophilization	:	• Sterility • Identification • Assay (virus concentration) • Abnormal toxicity • Water • Bovine serum albumin

S.2. Characterization:-

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

HISTORY OF OKA STRAIN OF VARICELLA VIRUS

Several vaccine strains of attenuated varicella virus have been developed and compared in both normal and immuno-suppressed people. The OKA strain has been shown to have the most desirable attribute of low virulence while inducing an adequate antibody response and protection against the disease.

The OKA strain is the only strain of varicella virus is currently considered suitable for vaccine production. The strain originated from a vesicle on a boy (OKA) who had chickenpox but who was otherwise healthy. The virus was isolated in human embryonic lung cells, and attenuated by serial passage in both human and guinea-pig embryo cells before further passages in human diploid cells. Clinical trials have been conducted in several countries. So far over 1 million people have been vaccinated with the virus, including both healthy children and adults and people with underlying disease, some with leukaemia. The vaccine has been shown to be safe, well tolerated and immunogenic in the doses used, which have ranged from 54 to 17000 plaque-forming units given by the subcutaneous route. There is evidence that some people who receive the OKA strain are protected from significant varicella disease in the absence of detectable sero-conversion; this may be due to cell-mediated immunity.

The virus isolated in primary human embryo lung cells was passaged 11 times at 34°C in cells from the same stored cell suspension. After a further 12 passages at 37°C in guinea pig embryo cells, the virus harvested was tested in children for its suitability. After three more passages at 37°C in human diploid cells the virus strain was considered to be suitable for vaccine production. Thus, from first isolation the virus was passaged through 26 sequential passages. A further two passages were made in human diploid cells (passage 28) which has been used for distribution from which the vaccine is being made.

Original preparation was contributed by the Research Foundation for Microbial Diseases, Osaka University to A.T.C.C. where further four passages were made in MRC-5; human diploid cells (ATCC CCL 171) to prepare lot 7W of VR-795. The virus have at no time have been passaged in continuous cell lines.

S.2.1.1 Physicochemical Characterization:-

VZV (Varicella zoster virus) is one of the eight human herpesvirus and is morphologically indistinguishable from HSV – 1, the prototype alphaherpesvirus. The VZV genome is linear, double – stranded DNA molecule 124,884 nucleotides in length with close homology to the HSV genome. The lipid envelop encloses the icosahedral nucleocapsid, which consists of 162 capsomeres. Virions are pleomorphic with a 150- to 200-nm diameter. In tissue culture, VZV produces a cytopathic effect in approximately 3days characterized by the formation of large multinucleated syncytia without the release of significant quantities of stable infectious virions.

The entire virus genome from 13 independent isolates has been sequenced. The VZV prototype consists of a long an short regions, each bounded by inverted and terminal repeat sequences. The long segment contains 104, 836 base pairs (bp) of unique DNA flanked by 88 – bp terminal repeat sequences. The short segment consists of 5232 bp of unique DNA flanked by 7320 bp repeat sequences. Analysis of the 124,884 bp VZV genome has identified 71 predicted open reading frames (ORF) numbered consecutively from the leftward and of the virus genome. ORF 62, 63 and 64 map within the internal repeat region of the short segment of the VZV genome and are duplicated (although in opposite orientation) as ORFs 71, 70 and 69 respectively, with in the terminal region. ORFs 42 and 45 may be exons from the same approximately 5.7 kbp primary transcript. There are 68 predicted unique VZV genes. Additionally, two novel VZV genes (ORFs 9a and 33.5) have been identified experimentally, indicating a potential coding capacity of 70 unique genes.

Alphaherpesvirus gene transcription is classified into three distinct kinetic groups: (1) immediate – early, (2) early, and (3) late. Innediate – early genes are transcribed in the absence of de novo protein synthesis and regulate transcription of early and late virus genes. The onset of early gene transcription precedes virus DNS replication. Transcription of early gene is induced by immediate – early proteins and early proteins, which are predominately involved in virus DNA replication and accumulate in the presence of inhibitors of DNA synthesis. Late proteins, which include the major virus structural proteins, are transcribed from progeny viral DNA, and their transcription is blocked by inhibitors of virus DNA synthesis.

During productive infection of cell in culture, transcripts mapping to all predicted VZV genes have been detected. PCR – based macroarrays developed to detect VZV transcription showed that major regions of gene transcriptions are not clustered, and instead are located throughout the virus genome. These results were confirmed in a study using oligonucleotide-based microarrays. Slight differences that were noted between the two array analysis are most likely caused by differences in virus strain and host cells.

S.2.1.2 Biological activity

Varicella-zoster virus (VZV) is a ubiquitous human alphaherpesvirus that causes varicella (chicken pox) and herpes zoster (shingles). Varicella is a common childhood illness, characterized by fever, viremia, and scattered vesicular lesions of the skin. As is characteristic of the alphaherpes viruses, VZV establishes latency in cells of the dorsal root ganglia. Herpes zoster, caused by VZV reactivation, is a localized, painful, vesicular rash involving one or adjacent dermatomes. The incidence of herpes zoster increases with age or immunosuppression. The VZV virion consists of a nucleocapsid surrounding a core that contains the linear, double-stranded DNA genome; a protein tegument separates the capsid from the lipid envelope, which incorporates the major viral glycoproteins. VZV is found in a worldwide geographic distribution but is more prevalent in temperate climates. Primary VZV infection elicits immunoglobulin G (IgG), IgM, and IgA antibodies, which bind to many classes of viral proteins. Virus-specific cellular immunity is critical for controlling viral replication in healthy and immune compromised patients with primary or recurrent VZV infections.

S.2.2. Impurities:-

Varicella Vaccine (Live) is a freeze dried preparation of attenuated OKA strain of Varicella-Zoster virus obtained by the propagation of the virus in MRC-5 human diploid cell culture.

Since the Varicella virus 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 final lot vaccine. The BSA concentration in the final lot vaccine remains less than 0.5 mcg per single human dose as per the specifications of Indian Pharmacopeia.

S.3. Control of Drug substance:-

S.3.1. Specification:-

A. SPECIFICATION FOR VIRUS HARVEST

S. No.	Name of the Test	Specifications as per Indian Pharmacopoeia
1.	Identification	The virus harvest contains virus that is identified as Varicella virus by serum neutralization in cell culture, using specific antibodies. Virus vaccine/sample when mixed with specific Human *Herpesvirus varicellae* antibodies, it is no longer able to infect susceptible cell cultures (MRC-5).
2.	Virus Concentration (Assay)	The combine mean estimate of the valid virus titer assay for the five samples tested in duplicate is not less than log $10^{4.5}$ PFU/ml.
3.	Extraneous agents	For the test to be valid at least 80% of the cell culture should be available and suitable for evaluation at the end of observation period. None of the cell cultures shows evidence of presence of extraneous agent attributable to the test material, no cytopathic changes or extraneous agents should be detected. No evidence of haemadsorbing agent is found.
4.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
5.	Mycobacteria	At the end of the incubation period no growth of mycobacteria occurs in any of the test media for the test sample, the preparation complies with the test. The test is valid if fertility of media is established by growth of mycobacteria in positive control.
6.	Test for absence of Mycoplasmas	If any container of liquid media shows bacterial or fungal contamination, repeat the test. If 7 days after inoculation, not more than one plate of solid media at each stage of the test has been contaminated with bacteria or fungi, or broken, that plate may be ignored provided that on immediate examination it shows no evidence of Mycoplasmas growth. If, at any stage of the test, more than one plate is contaminated with bacteria or

		fungi, or broken, the test is invalid and must be repeated. At the end of each incubation period microscopically examine inoculated solid media for the presence of Mycoplasmas. 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. Carry out the test for nutritive properties in the presence of the product to be examined. If growth of the test organisms is notably less than that found otherwise, the product contains inhibitory substances that must be neutralized (e.g. by dilution). The effectiveness of the neutralization process is checked by repeating the test for inhibitory substances after neutralization.

B. SPECIFICATION FOR CLARIFIED VIRUS HARVEST

S. No.	Name of the Test	Specifications as per Indian Pharmacopoeia
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
2.	Test for intact cell	Clarified virus harvest shall be negative for presence of intact cells.
3.	Virus Concentration (Assay)	The combine mean estimate of the valid virus titer assay for the five samples tested in duplicate is not less than log $10^{4.5}$ PFU/ml. The concentration of virus harvest is determined to monitor consistency of production and the dilution to be used for the final bulk vaccine.

C. SPECIFICATION FOR CONTROL CELL CULTURE

S. No.	Name of the Test	Specifications as per Indian Pharmacopoeia
1.	Test for observation for cytopathic agents	No cytopathic degeneration shall be observed in control cultures flasks throughout the time of incubation of the inoculated production cell cultures or for not less than 14 days beyond the time of incubation of the production cell cultures flasks whichever is longer. At least 80% of the control cell cultures survive/remain available to the end of the observation periods.
2.	Haemadsorbing viruses (agents)	No evidence of haemadsorbing agents is found.
3.	Test for identity (isoenzyme analysis)	The cell line shall be identified as human species (MRC-5) by comparing the corrected migrated distances of the test sample to known values for the species.
4.	Other extraneous	For the test to be valid at least 80% of the cell culture

	agents tests in cell cultures	should be available and suitable for evaluation at the end of observation period. None of the cell cultures shows evidence of presence of extraneous agent attributable to the test material, no cytopathic changes or extraneous agents should be detected. No evidence of haemadsorbing agent is found.

S.3.2. Stability summary and conclusions:-

Stability of Drug substance (Clarified Virus harvest)

The stability study was carried out on three lots of clarified virus harvest (bulk) of Varicella Vaccine (Live) I.P. (Bio Pox™), at recommended storage conditions (at or below -60°C). The conditions of study and number of batches considered are satisfactory. Based on the results of stability studies shelf life of 36 months was assigned for clarified virus harvest (bulk) at recommended storage condition of at or below -60°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 - 201009 (U.P.) INDIA
Phone : 0120-4157534, 4159857
Fax : 0120-4340219
E-mail : bmvaccine@yahoo.com
Website: www.biomed.co.in

P.1.2. Batch formula:-

Table A: Composition of the product per dose

S. No.	Ingredients	Quantity per dose 0.5 ml
1.	**Active ingredient:** Live attenuated Varicella virus (OKA strain) propagated in MRC-5 human diploid cell culture	≥2000 plaque forming units (PFU) or ≥ 3.3 $\log_{10}$ PFU/ dose*
2.	**Excipients** KH_2PO_4 K_2HPO_4 L-monosodium Glutamate EDTA Sucrose Human Normal Albumin Polygeline (Haemaccel®) **Diluent** Sterile Water for Injection I.P.	 0.25 mg 0.6 mg 0.4 mg 1 mg 37.5 mg 5 mg 5 mg 0.5 ml

*Targeted titre during blending is 3.95 log_{10} PFU/ dose.

P.1.3. Description of Manufacturing process & process control

The final bulk of Varicella Vaccine, Live I.P. is prepared by aseptic dilution of the clarified virus harvest based on the assay (virus concentration). The dilution of the clarified virus harvest for the preparation of final bulk is done by virus stabilizer containing salts, sucrose, polygeline (haemaccel).

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 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 slotted butyl stoppers, collected and loaded into freeze drier. The lyophilization cycle have been validated and the moisture content of consecutive six batches of final lot of Varicella Vaccine, Live 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 seal. The sealed vials are visually inspected, labeled, packed and stored at 2-8°C.

0.1ml overage has been taken considering the unrecoverable volume of the reconstituted vaccine (sticking to the vial or rubber stopper) and dead volume of the syringe and needle.

P.1.4. Control of critical steps & intermediate (name, dosage form)

PRODUCTION FLOW DIAGRAM OF VARICELLA VACCINE, LIVE I.P. FINAL LOT

Manufacturing Process	Control / Tests
Clarified virus harvest (stored at or below -60°C) ↓	<u>Clarified virus harvest</u> • Sterility • Test for intact cell • Virus concentration (Assay)
Aseptic dilution by virus stabilizer for preparation of final bulk ↓	<u>Final bulk</u> • Sterility
Filling, containerization and lyophilization ↓ Sealing ↓ Visual Inspection ↓ Labeling & Packing (Store at 2°C to 8°C)	<u>Final lot</u> • Sterility • Identification • Assay (virus concentration) • Abnormal toxicity • Water • Bovine serum albumin

P.2. Control of excipients:-

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

Human Normal Albumin is added as stabilizer for the Varicella Vaccine, Live I.P. The human normal albumin used for manufacture is of Indian Pharmacopoeia grade.

P.3. Control of Drug Product:-

P.3.1. Specification (s):

A. SPECIFICATION FOR FINAL BULK :

S. No.	Name of the Test	Specifications as Indian Pharmacopoeia
1.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.

B. SPECIFICATION FOR FINAL LOT :

S. No.	Name of the Test	Specifications as per Indian Pharmacopoeia
1.	Identification	Virus vaccine/sample when mixed with specific Human *Herpesvirus varicellae* antibodies, it is no longer able to infect susceptible cell cultures (MRC-5).
2.	Sterility	If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility.
3.	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.
4.	Bovine Serum Albumin	Not more than 0.5 µg per human dose.
5.	Water	Not more than 3.0 percent, determined by the semi-micro determination of water.
6.	Assay (Virus Concentration)	The combined mean estimate of the valid virus titer assay for the five vials /samples tested in duplicate of final lot vaccine is not less than 2000 PFU per vial.

P.3.2. Container Closure System:

Materials used for the final packing of Varicella Vaccine Live, 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:-

P.4.1 Stability summary & conclusion

Stability of Drug product (final lot of Varicella Vaccine, Live I.P.)

Stability studies real time (2-8°C) and at accelerated condition (20-25°C) have been conducted on three consecutive lots of Varicella Vaccine, Live 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 (2-8°C) for atleast 36 months. Based on the results of stability studies shelf life of 24 months was assigned for final lot of vaccine at recommended storage condition of +2 to +8°C.

Stability of Drug product (final lot of Varicella Vaccine, Live I.P.) after reconstitution with the diluent provided

Freeze dried vaccine after reconstitution with vaccine diluent was tested for stability at 20-25°C for up to 120 minutes. The test results prove good stability of the reconstituted vaccine at the conditions tested. The reconstituted vaccine at 20-25°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 Bio Pox™ 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 Bio Pox™ and list of equipments refer to Module 3 Point No. 3.2.A.

A.2. **Adventitious Agents Safety evaluation :-**

Varicella Virus Seed Lot(s)

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

The working virus seed lot is tested for neurovirulence in monkeys.

MRC-5 cells

The manufacturing working cell bank of MRC-5 cells are characterized for absence of extraneous agents using cell culture, retro viruses, animal inoculation, egg inoculation, mycoplasma, mycobacteria, bacteria and fungi.

Control Cell Culture

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

Virus harvest

Virus harvest of Varicella Vaccine, Live I.P. is characterized for absence of extraneous agents using cell culture, mycoplasma, mycobacteria, bacteria and fungi.

Clarified Virus harvest

Clarified virus harvest of Varicella Vaccine, Live I.P. is characterized for absence of extraneous agents by sterility test for bacteria and fungi.

Final Bulk

Final Bulk of Varicella Vaccine, Live I.P. is characterized for absence of extraneous agents by sterility test for bacteria and fungi.

Final Lot

Final lot of Varicella Vaccine, Live I.P. is characterized for absence of extraneous agents by sterility test for bacteria and fungi, Bovine Serum Albumin content by Rocket immunoelectrophoresis).