Draft New monograph for Comments and Inclusion in The Indian Pharmacopoeia

DRAFT NEW MONOGRAPH FOR COMMENTS

This draft new monograph contain text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in/ biologics-ipc@gov.in **before the last date for comments.**

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

Human Papillomavirus Vaccine (rDNA)

Human Papillomavirus vaccine (rDNA) is a sterile liquid preparation of purified virus like particles (VLPs) composed of the major capsid protein (L1) of one or more Human papillomavirus (HPV) genotypes; the antigens may be adsorbed on a mineral carrier such as Aluminium hydroxide or hydrated aluminium phosphate. The vaccine may also contain the other suitable adjuvant(s) such as 3-O-desacyl-4'-monophosphoryl lipid A. This preparation is whitish liquid in which the mineral carrier tends to settle down slowly on keeping but it is free from foreign particles or floccules. The recombinant yeast cells/ insect cells or E. coli/other suitable cell lines expressing the antigens are obtained by recombinant DNA technology (rDNA).

Production

General provisions

The vaccine shall have been shown to induce specific neutralizing antibodies in human. The production method shall have been shown to yield consistently vaccines comparable in Quality with the vaccine of proven clinical efficacy and safety in human.

The vaccine is produced by the expression of the viral genes coding for the capsid proteins in yeast / *E.coli* or in an insect cell /baculovirus expression vector system/ other suitable cell line, purification of the resulting VLPs and the rendering of these particles into an immunogenic preparation. The suitability and safety of the expression systems are approved by the competent authority. Production of the vaccine is based on a seed lot/cell bank system. Unless otherwise justified and authorised, the virus and cells used for vaccine production shall not have undergone more passages from the master seed lot/cell bank that was used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Reference preparation. A batch of vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. The reference vaccine is preferably stabilised and the stabilisation method shall have been shown to have no significant effect on the assay validity.

Characterisation

Characterisation of the VLPs is performed on lots produced during vaccine development, including the process validation batches. Characterisation includes protein composition, for example using techniques such as sodium dodecylsulfate polyacryl amide gel electrophoresis (SDS-PAGE) and Western blotting or mass spectrometry, peptide mapping and/or terminal amino acid sequence analysis. Morphological characteristics of the VLPs and degree of aggregation are determined to confirm the presence of the conformational epitopes that are essential for efficacy. VLP characterization may be done by methods like atomic force microscopy, transmission electron microscopy, dynamic light scattering, epitope mapping and reactivity with neutralizing monoclonal antibodies. In addition, the protein, lipid, nucleic acid and carbohydrate content are measured where applicable. The level of residual host-cell protein derived from insect cells meets acceptable safety criteria as set by the competent authority.

CELL BANKS AND SEED LOTS

Production in Recombinant yeast and Bacterial Only cell banks are prepared from the recombinant yeast and *Bacterial* cells. The prepared cell banks will be released for use after having satisfactorily characterized for identity, microbial purity, growth characteristics, viability and stability (as

applicable). Gene homogeneity is studied for the master and working cell banks. A full description of the biological characteristics of the host cell and expression vectors is given. The physiological measures used to promote and control the expression of the cloned gene in the host cell are described in detail. This includes genetic markers of the host cell, the construction, genetics and structure of the expression vector, the origin and identification of the gene that is being cloned. The nucleotide sequence of the gene insert and of adjacent segments of the vector and restriction-enzyme mapping of the vector containing the gene insert are provided. Data that demonstrates the stability of the expression system during storage of the recombinant working cell bank up to or beyond the passage level used for production is provided.

Production in an insect cell/baculovirus expression vector system

— *Insect cell substrate*. Only cell banks that have been satisfactorily characterized for identity, purity, growth characteristics, viability, stability, extraneous agents and tumorigenicity shall be used for production. Such characterization is performed at suitable stages of production in accordance with general chapters 2.7.2 Cell substrates for the production of vaccines for human use and 2.7.3 Tests for extraneous agents in viral vaccines for human use. Special attention is given to insect-borne viruses, in particular insect-borne potential human pathogens (e.g. arboviruses). Adventitious infectious agents of insect cells may be without cytopathic effect. Tests therefore include nucleic acid amplification techniques, and other tests such as electron microscopy and co-cultivation.

— *Recombinant baculovirus*. The use of the recombinant baculovirus vector is based on a seed-lot system with a defined number of passages between the original virus and the master and the working seed-lots, as approved by the competent authorities. The recombinant baculovirus expression vector contains the coding sequence of the HPV L1 antigen. Segments of the expression construct are analysed using nucleic acid amplification techniques in conjunction with other tests performed on the purified recombinant protein for assuring the quality and consistency of the expressed HPVL1 antigens. The recombinant baculovirus used in the production of HPV vaccines is identified by historical records, which include information on the origin and identity of the gene being cloned as well as the construction, genetics and structure of the baculovirus expression vector(s). The genetic stability of the expression construct is demonstrated from the baculovirus master seed up to at least the highest level used in production and preferably beyond this level.

Recombinant baculovirus seed lots are prepared in large quantities and stored at temperatures favourable for stability.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification. The master and working seed lots are identified by the HPV type of the inserted gene of origin, by an appropriate method such as nucleic acid amplification techniques (NAT) (2.8.1).

Virus concentration. The virus concentration of the master and working seed lots is determined to monitor consistency of production.

Extraneous agents (2.7.3). The working seed lot complies with the requirements for seed lots and control cells. Special attention is given to Spiro plasma spp. and insect-borne viruses, in particular insect-borne potential human pathogens (e.g. arboviruses).

PROPAGATION AND HARVEST

All processing of the cell banks and baculovirus seed lots and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Where justified and authorized for production in an insect cell/baculovirus expression vector system, a stored virus intermediate culture that complies with the 5 following tests may be used for virus propagation.

Identification. Each stored virus intermediate culture is identified by HPV type, by an immunological assay using specific antibodies or by a molecular identity test such as NAT (2.8.1).

Bacterial and fungal contamination. Each stored virus intermediate culture complies with the test for sterility (2.2.11), carried out using 10 ml for each medium.

Virus concentration. The virus concentration of each stored baculovirus intermediate culture is determined by a suitable method such as plaque assay or NAT (2.8.1) in order to monitor consistency of production.

Extraneous agents (2.7.3). Each stored virus intermediate culture complies with the tests for extraneous agents.

Control cells. The control cells of the production cell culture from which each stored virus intermediate is derived comply with a test for identity and with the requirements for extraneous agents (2.7.3).

Production in recombinant yeast/ Bacterial cells. Identity and microbial purity~~,~~ are determined at suitable production stages.

Production in an insect cell/baculovirus expression vector system. Insect cell cultures are inoculated with recombinant baculovirus at a defined multiplicity of infection as approved by the competent authority. Several single harvests may be pooled before testing. No antibiotics are added at the time of harvesting or at any later stage of manufacturing.

SINGLE HARVESTS

Only a single harvest or a pool of single harvests that complies with the following requirements may be used in the preparation of the purified monovalent antigen.

Identification (as applicable). Each single harvest is identified as the appropriate HPV type by immunological assay or by a molecular biology-based assay, for example hybridization or polymerase chain reaction (PCR).

Bacterial and fungal contamination. In case of production in an insect cell/baculovirus expression vector system the single harvest complies with the test for sterility (2.2.11). In case of production in yeast cells the single harvest is tested for culture purity by inoculation of suitable medium to ensure no growth other than the host organism.

Extraneous agents (2.7.3). In case of production in an insect cell/baculovirus expression vector system the single harvest complies with the tests for extraneous agents. Special attention is given to insect-borne viruses as mentioned under Cell banks and seed lots**.**

Control cells. In case of production in an insect cell/baculovirus expression vector system the control cells comply with a test for identification and with the requirements for extraneous agents (2.7.3). Special attention is given to insect-borne viruses as mentioned under Cell banks and seed lots.

PURIFIED MONOVALENT ANTIGEN

Harvests are purified using validated methods. When an insect cell/baculovirus expression vector system substrate is used, the production process is validated for its capacity to eliminate (by removal and/or inactivation) adventitious viruses and recombinant baculoviruses.

Only a purified monovalent antigen that complies with the following requirements may be used in the preparation of the final bulk. In agreement with the National Regulatory Authority (NRA), one or more of the tests mentioned below may be omitted if performed on the adsorbed monovalent antigen

Total protein
The total protein is determined by a suitable validated method. The content shall be within the limits approved for the particular product by NRA.

Antigen content and Identification (2.2.14)
The quantity and specificity of each antigen type is determined by a suitable immunochemical method (2.2.14) such as enzyme-linked immunosorbent assay (ELISA), radio-immunoassay (RIA), immunoblot (preferably using specific antibody directed against a protective epitope) or single radial diffusion. The antigen/protein ratio may be determined and shall be within the limits approved for the particular product by NRA. The test for antigen content may also serve as the identity test.

Antigenic purity
The purity of each purified monovalent antigen is determined by a suitable method, such as SDS-PAGE with quantification by densitometric analysis, the limit of detection being 1 per cent of impurities or better with respect to total protein. A reference preparation is used to validate each test. The protein purity is calculated as the ratio of the L1 protein-related bands relative to the total protein bands, expressed as a percentage. For the genotypes included in the vaccine, the value calculated for purity is within the limits approved for the particular product.

Percent of intact L1 monomer
The antigenic purity assay serves also to assess the integrity of the L1 monomer. The percent intact L1 monomer is the ratio of the intact L1 monomer to the total protein, expressed as a percentage. Percent intact monomer shall be within the limits approved for the particular product by NRA.

pH (2.4.24)
The pH is within the limits approved for the particular preparation.

VLP size and structure
The size and structure of the VLPs is established and monitored by a suitable method such as dynamic light scattering, Size Exclusion liquid Chromatography – High Performance liquid Chromatography (SEC-HPLC) and Transmission Electron Microscopy (TEM). The size shall be within the limits approved for the particular product by competent authority. This test may be omitted for routine lot release once consistency of production has been established, with approval of NRA.

Host-cell and vector-derived DNA (2.2.15)
Maximum 10 ng of DNA in a quantity of purified antigen equivalent to a single human dose of vaccine, determined in each monovalent purified antigen by sensitive methods.

Composition. The protein, lipid, nucleic acid and carbohydrate contents are determined, where

applicable.

Residual host-cell proteins
Tests for residual host-cell proteins are carried out by suitable validated method. The residual host cell protein content is within the limits approved for the particular product by NRA.

Residual Chemicals/reagents used for and purification or other phases of manufacturing: A test should be carried out to detect the presence of any reagents used during manufacture, using a method(s) approved by the NRA. The content is within the limits approved for the particular products. This test may be omitted for routine lot release upon demonstration that the process consistently eliminates the reagent from the purified monovalent antigen bulks.

Bovine serum albumin content (as applicable). If bovine serum is used in mammalian or insect cell cultures for production then residual bovine serum albumin content should be measured and a maximum permitted concentration should be set, and approved by the NRA.

Test for viral clearance (as applicable). When an insect or mammalian cell substrate is used for the production of HPV antigens, the production process should be validated in terms of its capacity to remove and/or inactivate adventitious viruses. This testing is performed during vaccine manufacturing development or as part of process validation and is not intended as an assessment for lot release. If a replicating viral vector such as a baculovirus is used then the production process should be validated for its capacity to eliminate (by removal and/or inactivation) residual recombinant virus.

Sterility (2.2.11)
Each purified monovalent antigen complies with the test, carried out using 10 ml for each medium.

ADSORBED MONOVALENT ANTIGEN
The purified monovalent antigens may be adsorbed onto an adjuvant such as an aluminium salt, in which case the adjuvant and the concentration used should be approved by the NRA. If an alternative or additional adjuvant such as MPL is used, this should also be approved by the NRA.

If a novel adjuvant is used that does not involve adsorption of the VLP to the adjuvant, the term “adjuvanted monovalent antigen bulk” may be used.

Only an adsorbed/ adjuvanted monovalent bulk that complies with the following requirements may be used in the preparation of the final bulk.

Sterility (2.2.11)
It complies with the test, carried out on adsorbed / adjuvanted monovalent bulk using 10 ml for each medium.

Bacterial Endotoxin(2.2.3)

Each adsorbed / adjuvanted monovalent bulk is tested for bacterial endotoxin test using a suitable validated method approved by competent authority.

Antigen content and Identification (2.2.14)
The quantity and specificity of each antigen type is determined by a suitable validated immunochemical method such as radio-immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), immunoblot (preferably using a specific antibody directed against a protective epitope) or single radial diffusion. The antigen/protein ratio is determined and is within the limits approved for the particular product by NRA. The test for antigen content may also serve as the identity test.

Mineral vehicle concentration. Where applicable, each adsorbed monovalent antigen is tested for the content of mineral vehicle. The content is within the limits approved for the particular product.

***ADSORBED3-O-DESACYL-4'-MONOPHOSPHORYL LIPID ABULK* (where Applicable)**
If 3-O-desacyl-4′-monophosphoryl lipid A is included in the vaccine it complies with the monograph 3-O-desacyl-4′-monophosphoryl lipid A. Where 3-O-desacyl-4′-monophosphoryl lipid A is adsorbed prior to inclusion in the vaccine, the adsorbed 3-O-desacyl-4′-monophosphoryl lipid A bulk complies with the following requirements.

Degree of adsorption of 3-O-desacyl-4′-monophosphoryl lipid A. The content of non-adsorbed 3-O-desacyl-4′monophosphoryl lipid A in the adsorbed 3-O-desacyl-4′monophosphoryl lipid A bulk is determined by a suitable method, for example gas chromatographic quantification of the 3-O-desacyl-4′-monophosphoryl lipid A fatty acids in the supernatant, evaporated to dryness, after centrifugation.

Sterility (2.2.11)
It complies with the test, carried out on adsorbed / adjuvanted monovalent bulk using 10 ml for each medium.

pH (*2.4.24)*
The pH shall be within the limits as approved the limits approved for the particular product by competent authority for the particular preparation.

FINAL BULK VACCINE
The final bulk vaccine is prepared directly from each purified monovalent antigen HPV type or purified monovalent antigen HPV type. An antimicrobial preservative, a mineral vehicle such as an aluminium salt and the adjuvant 3-*O*-desacyl-4'-monophosphoryl lipid A or any suitable adjuvant may be included in the formulation of the final bulk.

Only a final bulk that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than 85 percent and not greater than 115 percent of the intended content.

Sterility (2.2.11).The final bulk vaccine complies with the test, carried out using 10 ml for each medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and

Assay may be released for use. Provided that the test for antimicrobial preservative content (where applicable) has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot. If an *in-vivo* assay is carried out, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Adjuvants
If the vaccine contains an adjuvant, the amount is determined and shown to be within acceptable limits with respect to the expected amount. A suitable method for 3-*O*-desacyl-4′-monophosphoryl lipid A is, for example, gas chromatography.

Degree of adsorption
The degree of adsorption of each antigen and, where applicable, 3-*O*-desacyl-4′-monophosphoryl lipid A is assessed.

Identification
The vaccine is shown to contain Human Papillomavirus (L1) antigen by suitable immunochemical method such as enzyme-linked immunosorbent assay (ELISA). The in-vitro potency assay may serve to identify the vaccine. In addition, where applicable, the test for 3-O-desacyl-4′-monophosphoryl lipid A content also serves to identify the 3-O-desacyl-4′-monophosphoryl lipid A-containing vaccine.

Tests

Aluminium (2.3.9) maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

3-*O*-Desacyl-4′-monophosphoryl lipid A: minimum 80 per cent and maximum 120 per cent of the intended amount. Where applicable, determine the content of 3-*O*-desacyl-4′-monophosphoryl lipid A by a suitable method, for example gas chromatography (2.4.13).

Antimicrobial Preservative
Where applicable, determine the content of antimicrobial preservative by a suitable chemical or physio-chemical method. The amount is not less than the minimum amount shown to be effective and is not greater than 115 per cent of that stated on the label.

Sterility (2.2.11) Complies with the test for sterility.

Bacterial Endotoxin (2.2.3) Maximum 5 IU per single human dose. If the adjuvant prevents the determination of endotoxin, a suitable in-process test is carried out. However, where there is interference in the test – for example, because of the addition of an immunostimulant such as MPL – a test for pyrogens should be performed. The test is conducted until consistency of production is demonstrated, subject to the agreement of the NRA.

Osmolality. (2.4.23) The osmolality should be determined by a suitable method within the limit approved for particular preparation by NRA. The osmolality test may also be omitted for routine lot release upon demonstration of product consistency, subject to the approval of the NRA.

pH. (2.4.24) The pH is within the limits approved for the particular preparation.

Assay
The assay is performed by an *in vivo* test or an *in vitro* test having acceptance criteria established by correlation studies against an *in vivo* test.

***In vivo* test.** A suitable *in vivo* assay method consists of the injection of not fewer than 3 dilutions of the vaccine to be examined and of a reference vaccine preparation, using for each dilution a group of a suitable number of female mice of a suitable strain. The vaccine is diluted in a solution of sodium chloride containing the aluminium adjuvant used for the vaccine production. The mice are 6-8 weeks old at the time of injection, and each mouse is given a 0.5 ml injection. A preimmunisation serum sample is taken prior to inoculation, and a final serum sample is taken at a defined time between days 21 and 28. Assay the individual sera for specific neutralising antibodies against each HPV type by a suitable immunochemical method (2.2.14).
The test is not valid unless:

- for both the vaccine to be examined and the reference vaccine, the ED50 lies between the smallest and the largest doses given to the animals;
- the statistical analysis shows no significant deviation from linearity or parallelism;
- the confidence limits (P = 0.95) are within the limits approved for the particular product.

***In vitro* test.** Carry out an immunochemical determination (2.2.14) of each antigen genotype content. Enzyme-linked immunosorbent assay (ELISA) and radio-immunoassay (RIA) using specific antibodies specific for protection-inducing epitopes of the L1 protein have been shown to be suitable. Suitable numbers of dilutions of the vaccine to be examined and a manufacturer's reference preparation are used and a suitable model is used to analyse the data. For each type, the antigen content is within the limits approved for the particular product.

Protein content: The protein content should be determined. Alternatively, this may be calculated from an earlier process intermediate.

LABELLING
The label states:

- the amount of L1 proteins and the genotype of HPV contained in the vaccine;
- the cell substrate used for production of the vaccine;
- that the vaccine must not be frozen;
- number of doses, if the product is issued in a multiple-dose container;
- name and maximum quantity of any antibiotic present in the vaccine;
- name and concentration of any preservative added;
- name and concentration of any adjuvant and/or adsorbent;
- the temperature recommended during storage and transport;
- Manufacturing and expiry date;
- any special dosing schedules