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GUIDELINES TO ASSURE THE QUALITY, SAFETY AND EFFICACY OF RECOMBINANT HUMAN PAPILLOMAVIRUS VIRUS-LIKE PARTICLE VACCINES

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Introduction

WHO convened two meetings in Geneva, 23-24 March and 28 - 30 August, 2006, where scientific experts, regulatory professionals and other stakeholders met to develop guidelines for prophylactic human papillomavirus (HPV) vaccines. This document is intended to provide background and guidance to national regulatory authorities (NRAs) and vaccine manufacturers on the production, quality control and evaluation of the safety and efficacy of recombinant HPV virus-like particle (VLP) vaccines.

This document sets out the guidance on product manufacture and quality assessment in part A. In addition, guidance specific to the nonclinical and clinical evaluation of recombinant HPV vaccines is provided in Part B and Part C, respectively. This document should be read in conjunction with all relevant WHO guidelines including those on nonclinical (***1)*** and clinical evaluation (***2)*** of vaccines. The following text is written in the form of guidelines instead of recommendations. Guidelines allow greater flexibility than recommendations with respect to expected future developments in the field. This guidance is based on the experience of the products developed so far, as described below, and may need to be updated in view of future developments.

General considerations

HPV is a small, non-enveloped deoxyribonucleic acid (DNA) virus. The circular, double-stranded viral genome is approximately 8-kb in length. The genome encodes for 6 early proteins responsible for virus replication and 2 late proteins, L1 and L2, which are the viral structural proteins. L1 is the major structural protein. L1 proteins associate to form pentameric structures called capsomers. Mature virus particles are comprised of 72 capsomers arranged in icosahedral symmetry. The minor capsid protein, L2, is present in as many as 72 molecules per mature virus particle. L2 is not required for particle formation. HPV infection, replication and particle maturation occurs in the stratified squamous epithelia of skin and mucous membranes, with virus spread occurring by skin-to-skin contact.

Over 100 different types of HPV have been identified and molecularly characterized. These HPVs cause a variety of diseases in humans ranging from benign warts to cancer of the epithelia (including the cervix, vagina, vulva, anus and oropharynx). Those HPV types associated with the development of cancer are called high risk for oncogenicity. Other HPV types, such as HPV types 6 and 11 associated with genital warts, are considered low risk for oncogenicity.

The majority of HPV infections by both high and low risk types are often asymptomatic, self-limiting and resolve spontaneously, presumably due to the host immune response. In some instances, persistent infection by the high risk types may ultimately progress to invasive carcinoma at the site of infection,

mainly of the genital tract, if not detected and treated appropriately. The interval between the acquisition of HPV infection and malignant progression usually takes about 10 years or longer. High risk HPV types can be detected in virtually all cases of cervical cancer, and it is generally accepted that the persistent viral infection is necessary for the development of cancer (***3***). The basis for progression to invasive carcinoma is not well defined. However, environmental and physiological co-factors may increase the risk for cancer development in persistently infected persons.

The International Agency for Research on Cancer (IARC) has currently defined thirteen high risk HPV types that are associated with cancers in humans (***4***). Distribution and prevalence of these HPV types in cancer cases is generally consistent around the world. Two of the high risk HPV types, 16 and 18, account for approximately 70% of all cervical cancers globally (***4***). Most other genital cancers, such as cancers of the vagina and anus are also associated with persistent HPV infection. In addition, these HPVs are associated with a fraction of cancers of the vulva, penis, and oropharynx. The incidence of cervical cancer is significantly higher than all other HPV related cancers, and is the second most common cancer among women worldwide.

Low risk HPV types cause genital warts, recurrent respiratory papillomatosis (RRP), and low grade cervical dysplasia. The lifetime risk of genital warts exceeds 10%. While not malignant, these lesions are associated with physical and psychological morbidity. They are also difficult to treat. RRP is a devastating, albeit rare, disease that manifests as recurrent, rapidly growing benign laryngeal tumors that require frequent excision to prevent airway obstruction. HPV 6 and 11 are responsible for over 90% of genital warts and RRP cases, and 9 to 12% of low grade cervical dysplastic lesions.

Identification of a viral agent such as HPV as a major cause of diseases implies that prophylactic vaccines or interventions against the viral agent should prevent the disease(s) it causes. Initial studies in animal models showed that inoculation with species-specific papillomaviruses induced an immune response that conferred protection against homologous virus challenge. However, native papillomaviruses are not good substrates for vaccine development as they cannot be grown easily in culture. Subsequent studies were initiated on the production of viral particles from expression of the structural proteins in heterologous expression systems, such as yeast or baculovirus vectors. Results showed that expression of L1 alone led to the production of VLPs which morphologically resemble the authentic HPV virions but contain no viral DNA. These VLPs are produced by self-assembly of the L1 protein when expressed in a heterologous cell substrate and are the basis for the vaccines considered in this document. In animal studies, VLPs were shown to protect against high dose experimental infection by homologous virus. HPV VLPs are highly immunogenic in mice or rabbits, and the resulting antibodies have been shown to be neutralizing and type restricted when tested in a pseudovirion neutralization assay. Immunization with

denatured particles does not result in the production of neutralizing antibodies, or protect from experimental virus challenge, indicating that neutralizing epitopes are conformation dependent. Protection in animals has also been demonstrated through passive transfer of antibodies in serum.

Neutralizing antibodies are probably the primary mediator of this protection. L1 is not expressed in the basal keratinocytes in which infection is thought to be maintained and regression of established lesions was not observed after VLP vaccination. Therefore, it seems unlikely that cell-mediated immunity (CMI) is involved as a direct effector mechanism of protection (***5***).

The specific assays that have been developed to evaluate the immune response include: VLP-based enzyme immunoassay (EIA), competitive immunoassay with labeled neutralizing monoclonal antibodies, hemagglutination inhibition (HAI), and in vitro neutralization.

The development of these guidelines has been driven by the acquired experience with the two vaccines developed thus far. These vaccines are both made up of recombinant protein L1 VLPs and they contain adjuvant in order to stabilize the integrity of the L1 VLPs and also to enhance immunogenicity. The products differ in the types of HPV L1 proteins included as antigens, substrates used for production, adjuvant properties and in the final formulation. These two vaccines are:

1) A bivalent vaccine comprised of oncogenic HPV types 16 and 18 VLPs reassembled from L1 protein expressed and purified from insect cells infected with a recombinant baculovirus. This vaccine is formulated with a novel adjuvant, AS04, which contains aluminium hydroxide and monophosphoryl lipid A (MPL); and
2) A tetravalent vaccine comprised of the low risk HPV types 6 and 11 and the oncogenic HPV types 16 and 18. Type specific L1 proteins for this vaccine are expressed and purified from yeast cells containing L1 expression plasmids. The VLPs are adsorbed to an amorphous aluminium hydroxyphosphate sulfate-containing adjuvant.

It is possible that a vaccine produced in mammalian cells may be developed in the future.

Special considerations

There are several special considerations that need to be addressed in the manufacturing, non-clinical and clinical development of these vaccine products.

VLPs are complex biological products and will need to be assessed at various levels.

With respect to manufacturing and product quality the following items should be considered:

1) The bivalent vaccine expressed from recombinant baculovirus in insect cells is the first vaccine to be developed in this host expression system. Testing of this cell substrate may have some unique requirements;
2) A novel adjuvant which has not previously been experienced on a global scale is used in the formulation of the bivalent vaccine. The immunostimulant is MPL which is a detoxified form of lipid A derived from the lipopolysaccharide (LPS) isolated from bacterial cell walls of the Gram negative bacterium *Salmonella minnesota* R595. While detoxified, MPL was shown to retain the capacity of the natural LPS compound to act as an immunostimulant by potentiating cellular and humoral adaptive immune responses;
3) L1 protein in its native form is not glycosylated. For the two current vaccines glycosylation during production on a cell substrate is not an issue. HPV L1 VLP vaccines produced in new or different cell substrates should be assessed for glycosylation status;
4) Disassembly and reassembly of the L1 capsomers may contribute to purification of the product and lead to more stable VLPs;
5) Purified L1 VLP preparations will have to be characterized biochemically and immunologically, to determine L1 concentration, purity and assembly state; and
6) Current HPV vaccines are manufactured in single dose presentations without the addition of preservative. In the future, the availability of multi-dose vaccine vials would facilitate the adoption of innovative vaccination strategies targeting pre-adolescents and adolescents in developing countries. If these vaccines do not contain preservative, the use of such vaccine vials should be time-restricted as is the case of reconstituted vaccines such as Bacillus Calmette-Guérin (BCG) and measles-containing vaccines. If a preservative were to be added, the effect on antigenicity and immunogenicity must be assessed and known not to have an negative impact as has been observed with thiomersal (***6***).

With respect to the nonclinical studies it is critical that such studies demonstrate immunogenicity and the production of neutralizing antibodies.

With respect to clinical assessment of HPV VLP vaccines there are several critical considerations:

1) Since 90% of HPV related cancers are cervical cancers, the efficacy of the vaccines developed so far has been studied in sexually active women;
2) In order to obtain maximal benefit from these vaccines, the primary target population for immunization should consist of young adolescents prior to onset of sexual activity. Although the attack rate for HPV is high in the 5 to 10 years following sexual debut, most women remain naïve to vaccine HPV types during this time, and few have been infected with all vaccine HPV types;
3) Licensure of first generation vaccines requires a definitive demonstration of prophylactic efficacy with respect to cervical intraepithelial neoplasm (CIN) 2/3 and adenocarcinoma in situ (AIS) caused by vaccine HPV types;
5) Persistent infection (e.g. detection of the DNA of the same virion in cervicovaginal specimens collected on consecutive visits over a period of at least 12 months) may be an appropriate endpoint for second generation vaccines, including those with additional HPV types. At the time of preparing these Guidelines, however, there was no international consensus on a definition for HPV persistence based on detection of HPV DNA by restricted PCR; and
6) Once licensed, long term effectiveness evaluation of these vaccines should be integrated with current screening programs for cervical cancer.

Part A. Guidelines on manufacturing

A.1 Definitions

A.1.1 International name and proper name

The international name should be “Recombinant human papillomavirus virus-like particle vaccine” followed in parenthesis by the genotype specificity and the name of recombinant protein (e.g. genotype 16 and 18 L1 proteins). The proper name should be equivalent to the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the specifications elaborated below.

A.1.2 Descriptive definition

The recombinant HPV VLP vaccine is a sterile liquid vaccine preparation which contains purified VLPs composed of the recombinant major capsid proteins of one or more HPV genotypes (further referred to as "types"). The VLPs may be formulated with a suitable adjuvant. Such vaccines are for prophylactic use.

A.1.3 International reference preparations

International reference preparations based on recombinant HPV VLPs were not available when this Guidelines were prepared. However, reference reagents for use in the laboratory evaluation of the biological effects following vaccine administration to humans, such as antibody titers and viral DNA detection, are under development for HPV types 16 and 18. Some information can be found in the literature (***7-10)***.

A.1.4 Terminology

The definitions given below apply to this document only.

HPV L1 protein: The major structural protein of human papillomavirus, of which 360 molecules are found in the native virion associated in 72 pentameric capsomers.

L1 virus-like particle: A non-infectious, non-enveloped, icosahedral capsid particle which does not contain viral DNA and which is composed of regular arrays of L1 pentameric capsomers.

Parental yeast cell: Yeast host cell to be manipulated for the expression of protein(s) to give rise to a recombinant yeast production strain.

Recombinant baculovirus master seed lot: A quantity of recombinant baculovirus of uniform composition derived from an original baculovirus construct, processed at one time and passaged for a documented number of times.

Recombinant baculovirus working seed lot: A quantity of recombinant baculovirus of uniform composition, derived from the master seed lot by a limited number of passages. The recombinant baculovirus virus working seed lot may be used to prepare inoculum intermediates or alternatively to initiate the production of recombinant L1 proteins.

Inoculum intermediate: A quantity of recombinant baculovirus of uniform composition, derived from the working seed lot. The inoculum intermediate has a defined shelf-life. It is intended to be used to initiate the production of recombinant L1 proteins.

Cell bank: A collection of ampoules containing aliquots of a suspension of cells from a single pool of cells of uniform composition, stored frozen under defined conditions (typically <–60 °C for yeast, and in liquid nitrogen for insect or mammalian cell lines).

Master cell bank (MCB): A collection of containers containing aliquots of a suspension of cells from a single pool of cells of uniform composition, stored frozen under defined conditions (typically <–60 °C for yeast, and in liquid

nitrogen for insect or mammalian cell lines). The MCB is used to derive all working cell banks for the anticipated lifetime of the vaccine product.

Working cell bank (WCB): A collection of containers containing aliquots of a suspension of cells from a single pool of cells of uniform composition, derived from the MCB, stored frozen under defined conditions (typically <–60 °C for yeast, and in liquid nitrogen for insect or mammalian cell lines). One or more aliquots of the WCB are used for routine production of the vaccine. Multiple WCBs are made and used during the lifetime of vaccine product

Production cell culture: A cell culture derived from one or more containers of the WCB used for the production of vaccines.

End of production cells: A cell suspension containing the cells harvested at the end of culture/fermentation.

Adventitious agents: Contaminating microorganisms of the virus, or cell substrate or materials used in their cultures, that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced.

Fermentation cell paste: A suspension of cells harvested at the end of the yeast fermentation stored frozen (<-60°C).

Single antigen harvest: A cell-suspension containing the intended HPV antigens of one virus type harvested from cell cultures prepared from a single production run

Single harvest pool: A homogenous pool of multiple single harvests of the intended HPV antigens of one virus type, collected into a single vessel before clarification.

Purified monovalent antigen bulk: A batch of purified antigen of the same HPV type. Different batches of purified monovalent antigen bulks may be pooled before collection into a single vessel.

Adsorbed monovalent antigen bulk: A batch of purified monovalent antigen bulk adsorbed on an aluminium containing adjuvant. Different batches of adsorbed monovalent antigen bulks may be pooled before collection into a single vessel.

Adjuvant: A vaccine adjuvant is a component that potentiates the immune response to an antigen and/or modulates it towards the desired immune responses.

Final vaccine bulk: The formulated bulk present in the container from which the final containers are filled. The final bulk may be prepared from one or more adsorbed monovalent antigen bulks and may contain VLP antigens from one or multiple HPV virus types.

Filling lot (final vaccine lot): A collection of sealed final containers of vaccine that is homogeneous with respect to the risk of contamination during the filling process. A filling lot must therefore have been filled or prepared in one working session.

A.2 General manufacturing recommendations

The general manufacturing requirements contained in *Good manufacturing practices for biological products* (***11)*** should apply to the establishment of manufacturing facilities for recombinant HPV VLP vaccines, with the addition of the following:

- Production steps involving manipulations of recombinant HPV L1 VLP types should be conducted at a biosafety level consistent with the recombinant production microorganism;

- Quality control procedures should be in place to ensure segregation of different HPV L1 VLP types during bulk manufacturing steps. Sufficient cleaning validation and product changeover data should be available; and

- The antigen manufacturing process should be validated to demonstrate production consistency. Typically, three consecutive lots per HPV type are required. However, if one or more HPV types use the same manufacturing process, validation of all processes with at least one type may be acceptable. The assessment of manufacturing consistency should include evaluation of critical quality parameters and their corresponding attributes. Examples of process quality attributes are nucleic acid and host cell protein clearance or cumulated population doubling level and examples or process key operating parameters is column loading. The process validation antigen batches should show compliance with the pre-established antigen quality control specifications for the HPV antigen such as antigen identity and antigen purity (see section A.5).

A.2.1 Characterization of the antigen

Characterization of HPV antigen is performed on lots produced during vaccine development, including the process validation batches.

The protein composition should be established by techniques such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions or mass spectrometry. The bands should be identified by sensitive staining techniques and where possible by specific antibodies or mass spectrometry to confirm the presence of the expected products of the L1 protein. The identity of the protein should be established by peptide mapping and/or terminal amino acid sequence analysis.

Since it is known that conformational epitopes are essential for efficacy, it is essential that the morphological characteristics of the VLPs and degree of aggregation should be determined. In addition, the protein, lipid, nucleic acid and carbohydrate content should be measured when applicable. VLP characterization may be done by atomic force and transmission electron microscopy, dynamic light scattering, epitope mapping and reactivity with neutralizing monoclonal antibodies.

The level of residual host cell protein derived from insect cells and/or a novel cell substrate should meet acceptable safety in nonclinical and clinical studies (see Parts B and C).

A.3 Control of source materials

A.3.1 Cell cultures for antigen production

The use of any cell line should be based on a cell bank system. Only cells that have been approved and registered with the national regulatory authority should be used to produce HPV L1 protein. The national regulatory authority should be responsible for approving the cell bank. Appropriate history of the cell bank should be provided.

A.3.1.1 Yeast cells

The characteristics of the recombinant production strain (host cell in combination with the expression vector system) should be fully described and information given on the absence of adventitious agents and on gene homogeneity for the master and working cell banks. A full description of the biological characteristics of the host cell and expression vectors should be given. The physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail. This should include genetic markers of the host cell, the construction, genetics and structure of the expression vector and 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 should be provided as required by the national control authority**.**

A.3.1.2 Insect cells

If insect cells are used for production of recombinant HPV L1 VLP vaccines, the use of insect cell substrate should be based on a cell bank system. The cell substrates and cell banks should conform with *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12,13)***, as appropriate to insect cells, and should be approved by the national regulatory authority.

The maximum number of passages (or population doublings) allowable between the MCB, the WCB and the production cells should be approved by the national regulatory authority. Additionally, the MCB or WCB cells should be propagated to or beyond the maximum production level and be examined for tumorigenicity in an animal test system and for the presence of retroviruses and arthropod-borne viruses.

The MCB is made in sufficient quantities and stored in a secure environment and is used as the source material to make manufacturers WCB. In normal practice a MCB is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national regulatory authority, at which point the cells are combined to give a single pool distributed into ampoules and preserved cryogenically to form the WCB.

The manufacturers working cell bank is used for the preparation of production cell culture, and thus for production of HPV L1 antigen batches.

A.3.1.3 Other Cell Substrates

If other host cells are used, the cell substrates and cell banks should conform with *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12,13)*** where appropriate, and should be approved by the national regulatory authority.

A.3.2 Cell culture medium

If serum is used for the propagation of cells, it should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, according to the requirements given in Part A, sections 5.2 (***14)*** and 5.3 (***15)***of *Requirements for biological substances no. 6* and from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of *Recommendations for production and control of poliomyelitis vaccine (oral)* (***16)***.

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera. As an additional monitor of quality, sera may be examined for freedom from phage and endotoxin. Gamma-irradiation may be used to inactivate potential contaminant viruses.

The acceptability of the source(s) of any components of bovine, porcine, sheep or goat origin used should be approved by the national regulatory authority. These components should comply with current WHO guidelines in relation to animal transmissible spongiform encephalopathies (***17)***.

If trypsin is used for preparing cell cultures and aiding in virus infection, it should be tested and found free of bacteria, fungi, mycoplasmas and infectious viruses, especially bovine or porcine parvoviruses, as appropriate. The methods used to ensure this should be approved by the national regulatory authority. The trypsin should be gamma irradiated if possible.

Human serum should not be used.

However, human serum albumin may be used. If used, it should meet *Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (requirements for biological substances no. 27)* (***18***), as well as current guidelines in relation to human transmissible encephalopathies (***17***).

Penicillin and other beta-lactams should not be used at any stage of the manufacture because of their nature as highly sensitizing substances.

Other antibiotics may be used in the manufacture provided that the quantity present in the final product is acceptable to the national regulatory authority.

Non-toxic pH indicators may be added, e.g. phenol red in a concentration of 0.002%. Only substances that have been approved by the national regulatory authority may be added.

A.3.3 Tests on master and working cell banks

A.3.3.1 Yeast cells

Master and working cell banks should be tested for the absence of adventitious bacteria, fungi and mycoplasmas according to Part A, section 5.2 of *Requirements for biological substances no. 6* (***14***) or by a method approved by the national regulatory authority.

Cells must be maintained in a frozen state that allows recovery of viable cells without alteration of genotype. The cells should be recovered from the frozen state, if necessary in selective media such that the genotype and phenotype consistent with the unmodified host and unmodified recombinant DNA vector are maintained and clearly identifiable. Cell banks should be identified and fully characterized by means of appropriate tests.

Data that demonstrate the stability of the expression system during storage of the recombinant WCB up to or beyond the passage level used for production should be provided and approved by the national regulatory authority. Any instability of the expression system occurring in the seed culture or after production-scale run should be documented.

A.3.3.2 Insect cells for recombinant baculovirus expression system

Tests on the master and working cell banks should be performed in accordance with *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12***). In addition, it is important to show that the cell banks are free of bacteria, fungi, mycoplasmas, mycobacterium spp, and adventitious agents relevant to the species used in its derivation. For insect cell lines, special emphasis is put on potential insect-borne human pathogens (e.g. arboviruses). Cell banks should be assessed for absence of adventitious agents that may have been present during production, including those that may be present in the source materials used at each of these stages.

Insect viruses have not been well characterized compared to other potential adventitious agents. It should be borne in mind that infection of insect cells may be without cytopathic effect. Tests may include PCR, and other tests such as electron microscopy and co-cultivation. The specificity and sensitivity of assays should be defined and approved by the NRA.

Viruses of invertebrates include ascoviruses, baculoviruses, birnaviruses, dicistroviruses, iridoviruses, metaviruses, nimaviruses, nodaviruses, parvoviruses, polydnaviruses, poxviriuses, pseudoviruses, reoviruses, and tetraviruses. However, their relevance to the species of origin of insect cells used in cell bank and production was largely not known at the time of preparing these Guidelines. Arboviruses (arthropod-borne viruses) of vertebrates include bunyaviruses, flaviviruses, togaviruses, reoviruses (especially, orbiviruses), rhabdoviruses, and asfaviruses. Other adventitious agents may include retrotransposon and TSE agents if bovine serum used ever.

Full characterization may be performed on either the master cell bank or on the working cell bank (***12***).

A.3.3.3 Other cell substrates

Tests on the master and working cell banks are performed in accordance with *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12***) and *Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology* for recombinant cells (***19***).

A.3.4 Recombinant baculovirus master seeds and working seeds

The recombinant baculovirus expression vector contains the coding sequence of the recombinant HPV protein antigen. Segments of the expression construct should be analysed using nucleic acid techniques in conjunction with other tests performed on the purified recombinant protein for assuring the quality and consistency of the expressed HPV L1 antigens.

The recombinant baculovirus used in the production of HPV vaccines should be identified by historical records, which will include information on the origin and identity of the gene being cloned as well as the construction, the genetics and structure of the baculovirus expression vector(s). The genetic stability of the expression construct should be demonstrated from the baculovirus master seed up to at least the highest level used in production but preferably beyond this level (***19,20***).
The production of vaccine should be based on the recombinant baculovirus master seed lot and working seed lot system. Recombinant baculovirus seed lots should be stored in a dedicated temperature-monitored refrigerator at a temperature that ensures stability and security.

Seed lots of recombinant baculovirus used in the production of HPV antigens should be identified by historical records, which should include information on their origin. Only recombinant baculovirus seed lots that are approved by the national regulatory authority should be used. The recombinant baculovirus master seed lot is made in sufficient quantities and stored in a secure environment and is used as the source material to make the manufacturers recombinant baculovirus working seed lots. Either the virus master seed lots or the virus working seed lots should be fully characterized and be tested extensively for adventitious agents, and approved by the national regulatory authority.

The recombinant baculovirus master seed lot also serves as a benchmark from which to compare virus produced by subsequent passage in cell culture.

The manufacturers recombinant baculovirus working seed lot is used for the production of HPV antigen batches and is prepared from the master recombinant baculovirus seed lot. It is recommended that a large lot of virus working seed be set aside as the basic material that the manufacturer should use for the preparation of each batch of vaccine. The recombinant baculovirus working seed lot should be prepared by defined number of passage from the recombinant baculovirus master seed lot by a method and a passage level from the original virus seed and approved by the NRA. Once the passage level of the working seed lot is established, it may not be changed without approval from the national regulatory authority.

A.3.4.1 Control tests on recombinant baculovirus master and working seed lots

A.3.4.1.1 Identity

Each baculovirus master and working seed lot should be identified by HPV type of the inserted gene of origin by an appropriate method such as PCR. The tests should be approved by the national regulatory authority.

A.3.4.1.2 Sterility tests for bacteria, fungi and mycoplasmas

Each recombinant baculovirus seed lot should also be tested for bacterial, fungal, and mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 (***14)*** and 5.3 (***15)*** of *Requirements for biological substances no. 6*. In addition, the recombinant baculovirus seed lot should be tested for insect mollicutes (mycoplasma) such as spiroplasma, entomoplasma and mesoplasma.

A.3.4.1.3 Tests for adventitious viruses

Each recombinant baculovirus seed lot should be tested in cell cultures for adventitious viruses appropriate to the origin and the passage history of the seed baculovirus. For tests on recombinant baculovirus-permissive detector cells, neutralization of baculovirus is necessary. Antisera used for this purpose should be shown to be free from antibodies that may neutralize specific adventitious viruses being tested for. The inoculated cells should be examined microscopically for cytopathic changes. However, infection of insect cells may not result in cytopathic effect. Tests may include PCR, electron microscopy and co-cultivation. At the end of the examination period, the cells should be tested for haemadsorbing viruses. It is important to show that recombinant baculovirus seeds are free of adventitious agents relevant to the species used in their derivation with special emphasis on potential insect-borne human pathogens (e.g. arboviruses). The specificity and sensitivity of assays should be defined and approved by the NRA

In general, recombinant baculovirus seeds should be assessed for absence of adventitious agents that may have been present during their production, including those that may be present in the source materials used at each of the production stages. Each virus master or working seed lot should also be tested in animals that may include guinea pigs and mice. For test details refer to *Requirements for measles, mumps and rubella vaccines and combined vaccines (live)* (***21)***.

A.3.4.1.4 Test for mycobacterium spp

Each recombinant baculovirus seed lot should be tested for mycobacterium spp. The test method and specifications should be approved by the NRA.

A.3.4.1.5 Tests on control cells used for production of seeds

Tests on control cell cultures should be undertaken as detailed in section A.4.2.1.

A.3.4.1.6 Recombinant baculovirus concentration

Each recombinant baculovirus seed lot will be assayed for infectivity in a sensitive assay in an insect cell culture system. The detailed procedures for carrying out the tests and for interpreting the results should be those approved by the national regulatory authority.

A.4 Control of HPV antigen vaccine production

A.4.1 Control of HPV antigen vaccine production up to single harvest in yeast expression system

A.4.1.1 Microbial purity

Microbial purity in each fermentation vessel should be monitored at the end of the production run by methods approved by the national regulatory authority.

Any agent added to fermentor or bioreactor in purpose to feed cells or to induce / increase cell density should be approved by the national regulatory authority.

A.4.2 Control of HPV antigen vaccine production up to single harvest in recombinant baculovirus system in insect cells

Cell cultures are inoculated with recombinant baculovirus at a defined multiplicity of infection. After adsorption, the cell cultures are fed with maintenance medium and incubated within a defined temperature range and for a defined period of time.

The range of multiplicity of infection (MOI), temperature, pH and incubation period will depend on the insect cell substrate and the recombinant baculovirus strain specifics. A defined range for the MOI should be established by the manufacturer and be approved in the market authorization by the national regulatory authority.

A single harvest is harvested within a defined time period post inoculation. Several single harvests may be pooled. If multiple single harvests are pooled, each single harvest should be sampled for testing, stabilized and stored under suitable conditions until pooling. No antibiotics should be added at the time of harvesting or at any later stage of manufacturing. Samples of single harvest pools should be taken for testing and stored at a temperature of -60 ^{o}C or below.

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A.4.2.1 Tests on control cells culture

When the cell suspension is used to prepare cell cultures for expression of the HPV antigens, an amount of processed cell suspension equivalent to at least 5% or 500 ml of cell suspension, whichever is greater, should be used to prepare control cultures of uninfected cells. If bioreactor technology is used, the size and treatment of the cell sample to be examined should be approved by the national regulatory authority.

The control cell cultures should be examined microscopically for the morphological changes of the cells attributed to the presence of adventitious agents for at least 14 days after the day of inoculation of the production cultures

or at the time of final virus harvest if this is later. The control cell cultures should be incubated essentially under similar conditions as the production cultures with agreement of the national regulatory authority. For insect cells, the above incubation time may not apply due to the specificities of cells cultivated in suspension but should be not less that the time of collection of the single harvest. At the end of the examination period, fluids collected from the control cell culture from each single harvest should be tested for the presence of adventitious agents as described below. Samples that are not tested immediately should be stored at -60 ^{o}C or below.

If any test shows evidence of the presence of adventitious agents in control cell cultures, the single harvests prepared these cultures should not be used for HPV antigen production.

For the test to be valid, at least 80% of the control cells should have survived by the end of the test period.

A.4.2.1.1 Tests for haemadsorbing viruses

At the end of the observation period, cells comprising no less than 25% of the control cells should be tested for the presence of haemadsorbing viruses, using guinea-pig red blood cells. If the red blood cells have been stored, the duration of storage should not have exceeded 7 days, and the temperature of storage should have been in the range of 2-8 ^{o}C.

In some countries, the national regulatory authority requires that additional tests for haemadsorbing viruses will be performed using other species of red blood cells including those from humans (blood group O), monkeys, and chickens (or other avian species). All tests should be read after incubation for 30 minutes at 0-4 ^{o}C, and again after a further incubation for 30 minutes at 20-25 ºC. The test with monkey red blood cells should be read once more after an additional incubation for 30 minutes at 34-37 ^{o}C.

For cells cultivated in suspension, the test for presence of haemadsorbing viruses is not technically feasible. A test for presence of haemagglutinating agents using guinea-pig red blood cells is therefore required.

For the tests to be valid, at least 80% of the culture cells should have survived by the end of the test period.

A.4.2.1.2. Tests for other adventitious agents

At the end of the observation period, a sample of the pooled fluid from each group of control cell cultures should be tested for adventitious agents. For this purpose, 10 ml of each pool should be tested in the same cells as those used for the production of virus, but not the same batch of cells, and additional 10 ml samples

of each pool should be tested in human cells and at least one other sensitive cell system.

Each sample should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cells should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of the cell cultures should remain uninoculated as a control.

The inoculated cultures should be incubated at the appropriate temperature and should be observed for cytopathic effects for a period of at least 14 days.

For the tests to be valid, at least 80% of the culture cells should have survived by the end of the test period.

Furthermore, some national regulatory authorities require that these cells should be tested for the presence of haemadsorbing viruses.

A.4.2.1.3 Test for identity of insect cells

At the production level, the cells should be identified by means of tests approved by the national regulatory authority. Suitable methods are, but not limited to, biochemical tests (e.g. isoenzyme analyses), cytogenetic tests (e.g. for chromosomal markers), and tests for genetic markers (e.g. DNA fingerprinting).

A.4.3 Control of HPV antigen vaccine production up to single harvest in mammalian cells

Tests on control cells for identity and adventitious agents should be performed in accordance with *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12***).

A.4.4 Control of single harvests

A.4.4.1 Storage and intermediate hold times

Prior to and during the purification process, the cell suspension should be maintained under conditions shown by the manufacturer to retain the desired biological activity. Hold times should be approved by the national regulatory authority.

A.4.4.2 Tests on single harvest or single harvests pool

If appropriate, tests may be done on single harvest or on single harvests pools. If the tests are done on the single harvests pool, the protocol should be approved by the national regulatory authority.

A.4.4.2.1 Sampling

Samples required for the testing of single harvests or single harvests pools should be taken immediately on harvesting prior to further processing. If the tests for adventitious agents as described in Part A, are not performed immediately, the samples taken for these tests should be kept at a temperature of -60 ºC or below and subjected to no more than one freeze-thaw cycle.

A.4.4.2.2 Sterility tests

Each single harvest or single harvests pool should be shown to be free from bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2 of *Requirements for biological substances no. 6* (***14***).

In addition to sterility tests for bacteria and fungi, each single harvest or single harvests pool should be shown to be free from mycoplasmal contamination by appropriate tests as specified in Part A, section 5.3 of *Requirements for biological substances no. 6* (***15***) if insect or mammalian cells are used in production.

A.4.4.2.3 Test for identity of HPV types

Each single harvest or single harvests pool should be identified as the appropriate HPV type by immunological assay or by a molecular biology-based assay, e.g. hybridization or PCR. The tests should be approved by the national regulatory authority. The identity can instead be confirmed as part of testing of the purified antigen.

A.4.4.2.4 Tests for adventitious agents if insect or mammalian cells are used in production

For the purposes of the recommendations set out in this section of Part A, the volume of each single harvest or single harvest pool taken for neutralization (if applicable) and testing should be at least 10 ml and should be such that a total of at least 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater.

Each single harvest or single harvests pool should be tested for adventitious viruses in cell cultures selected for their appropriateness to the origin and the passage history of the insect cell substrate and recombinant baculovirus. Neutralization of recombinant baculovirus is necessary for tests on baculovirus-sensitive insect cells because the virus is cytopathic. Antisera used for this purpose should be free from antibodies that may neutralize the adventitious viruses being tested for. The cells inoculated should be observed microscopically for cytopathic changes. At the end of the observation period, the cells should be tested for haemadsorbing viruses.

Additional testing for specific adventitious viruses may be performed for example using PCR amplification techniques.

If mammalian cells are used, tests for adventitious agents for single harvests should be performed in accordance with *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12***).

A.5 Control of purified monovalent antigen bulk

The purification process can be applied to a single antigen harvest, a part of a single antigen harvest or a pool of single antigen harvests. The maximum number of harvests that may be pooled should be approved by the national regulatory authority. The antigen is purified before adsorption to the mineral vehicle. Adequate purification may require several purification steps based on different principles and may involve disassembly and re-assembly of VLPs. The entire process (sequence of methods) used for the purification of the VLPs should be appropriately validated as described in section A2 and approved by the national regulatory authority. Any agent added to purification process, such as Benzonase, should be documented.

The monovalent antigen purified bulk can be stored under conditions shown by the manufacturer to retain the desired biological activity. Intermediate hold times should be approved by the national regulatory authority.

A.5.1 Tests on the monovalent antigen purified bulk

Monovalent antigen purified bulks should be tested according to the tests listed below. Some tests may be omitted if performed on the adsorbed monovalent antigen bulk. All quality control release tests and specifications for monovalent antigen purified bulk, unless otherwise specified, should be validated and approved by the national regulatory authority.

A.5.1.1 Identity

Each monovalent antigen purified bulk should be identified as the appropriate HPV antigen type by immunological assay.

A.5.1.2 Purity

The degree of purity of each monovalent antigen purified bulk should be assessed by suitable methods. One suitable method of analysing the proportion of potential contaminating proteins in the total protein of the preparation is separation of the proteins by polyacrylamide gel electrophoresis under reducing denaturing conditions. Individual gels should be stained using a suitable dye such as

coomassie blue. The protein in each band should be quantified by densitometric analysis.

A.5.1.3 Protein content

Each monovalent antigen purified bulk should be tested for the total protein content using a suitable method such as the micro-Kjeldahl method, the Lowry technique or another suitable method.

A.5.1.4 Antigen content

The antigen content of the purified preparation may be measured by an appropriate method which is type specific until production consistency is demonstrated**.** This test may be omitted if a test for antigen content is performed on the adsorbed monovalent antigen bulk

The ratio of antigen content to protein content may be determined on each monovalent antigen purified bulk.

A.5.1.5 Sterility tests for bacteria and fungi

Each monovalent antigen purified bulk should be tested for bacterial and fungal sterility according to Part A, section 5.2 of *Requirements for biological substances no. 6* (***14)***, or by a method approved by the national regulatory authority. This test can alternatively be performed on the related adsorbed monovalent antigen bulks should the purified bulk not be held prior to adsorption shelf life be short .

A.5.1.7 Percent intact L1 monomer

The percent intact L1 protein may be assessed by suitable methods until production consistency is demonstrated.

A.5.1.8. VLP size and structure

The size and structure of the VLPs are to be established and monitored until production consistency is demonstrated. The specification should be approved by the NRA.

A.5.1.9 Tests for agents used during purification or other phases of manufacture

A test should be made for the presence of any potentially hazardous agents used during manufacture. This test may be omitted for routine lot release upon demonstration that the process consistently eliminates the agent from the monovalent antigen purified bulks.

A.5.1.10 Tests for residuals derived from the antigen expression system

The amount of residuals derived from the antigen expression system (e.g. DNA or host cell proteins) should be determined in each monovalent antigen purified bulk by sensitive methods. The level of host cell DNA should not exceed the maximum level cited in *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***12***).

These tests may be omitted for routine lot release upon demonstration that the process consistently eliminates the tested residuals from the monovalent antigen purified bulks.

A.5.1.11 Albumin content

If animal serum is used in mammalian or insect cell cultures for production, residual albumin content should be measured.

A.5.1.12 Test for viral clearance

When an insect or mammalian cell substrate is used for the production of HPV antigens, the production process should be validated for its capacity to eliminate (by removal and/or inactivation) adventitious viruses as described in the ICH Q5A guidelines (***22***). This testing is performed during vaccine manufacturing development and/or process validation and is not intended for batch release.

If a replicating viral vector, e.g. baculovirus, is used, the production process should be validated for its capacity to eliminate (by removal and/or inactivation) residual recombinant virus.

A.6 Adsorbed monovalent antigen bulk

A.6.1 Addition of adjuvant (mineral vehicle)

The antigens may be adsorbed onto a mineral vehicle such as aluminium salt. In that case, the mineral vehicle and its concentration used should be approved by the national regulatory authority.

A.6.2 Storage

Until the bulk is formulated into the final bulk, the suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity. Hold times should be approved by the national regulatory authority.

A.6.3 Tests on adsorbed monovalent antigen bulk

All tests and specifications for absorbed monovalent antigen bulk, unless otherwise specified, should be approved by the national regulatory authority.

A.6.3.1 Sterility tests for bacteria and fungi

Each adsorbed monovalent antigen bulk should be tested for bacterial and fungal sterility according to Part A, section 5.2 of *Requirements for biological substances no. 6* (***14)***, or by an alternative method approved by the national regulatory authority.

A.6.3.2 Bacterial endotoxins

Each adsorbed monovalent antigen bulk should be tested for bacterial endotoxins.

A.6.3.3 Identity

Each adsorbed monovalent antigen bulk should be shown to contain the appropriate HPV antigen by a type-specific assay. The antigen content test may also serve as the identity test.

A.6.3.4 Mineral vehicle concentration

Adsorbed monovalent antigen bulk may be assayed for the content of the mineral vehicle until production consistency is demonstrated.

A.6.3.5 Degree of adsorption

The degree of adsorption (completeness of adsorption) of each adsorbed monovalent antigen purified bulks should be assessed. This test may be omitted upon demonstration of process consistency.

A.6.3.6 pH

The pH value of the adsorbed monovalent antigen bulk may be monitored until production consistency is demonstrated.

A.6.3.7 Antigen content

The antigen content of the adsorbed monovalent antigen bulk should be measured with appropriate methods, unless tested at the purified antigen bulk stage.

A.7 Final vaccine bulk

The final bulk should be aseptically prepared by combination of adsorbed monovalent antigen bulks which pass the tests specified in section A.6.3. The antigen concentration in the final formulation should be sufficient to ensure the

dose which is consistent with that shown to be safe and effective in human clinical trials. Should an adjuvant (mineral vehicle or immunostimulant) be added to the vaccine formulation, the adjuvant and the concentration used should be approved by the national regulatory authority.

The operations necessary for preparing the final bulk vaccine lot should be conducted in such a manner as to avoid contamination of the product. In preparing the final bulk vaccine, any substances such as diluents, stabilizers or adjuvants that are added to the product should have been shown to the satisfaction of the national regulatory authority not to impair the safety and efficacy of the vaccine in the concentration used. Until the bulk is filled into containers, the final bulk suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity.

A.7.1 Tests on the final bulk vaccine

All tests and specifications for final bulk vaccine, unless otherwise specified, should be approved by the national regulatory authority.

A.7.1.1 Sterility tests for bacteria and fungi

Each final bulk vaccine should be tested for bacterial and fungal sterility according to Part A, section 5.2 of *Requirements for biological substances no. 6* (***14)***, or by a method approved by the national regulatory authority.

A.7.1.2 Adjuvants

Each final bulk should be assayed for the content of adjuvants. The method used and permitted concentrations should be approved by the national regulatory authority.

Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.

Suitable tests for immunostimulants such as MPL are e.g. gas chromatography.

Tests for adjuvants may be conducted on each final vaccine lots derived from the final bulk.

A.7.1.3 Degree of adsorption

The degree of adsorption (completeness of adsorption) of each antigen present in each final vaccine bulk should be assessed.

This test may be omitted upon demonstration of the process consistency or if performed on the final vaccine lot.

A.7.1.4 Preservative content

The final bulk may be tested for the presence of preservative if added. The method used and the permitted concentration should be approved by the national regulatory authority.

A.7.1.5 Potency

If an in vivo potency test is used, this test may be performed on the final bulk. The method for detection of antibody and the analysis of data should be approved by the national regulatory authority. The vaccine potency should be compared with that of a reference preparation and the national regulatory authority should determine limits of potency. The national regulatory authority should approve the reference preparation used.

This test may be conducted on each final vaccine lot derived from the final bulk. If an in vitro potency test is performed, it should be performed on every lot of final vaccine lot

A.8 Filling and containers

The requirements concerning filling and containers given in *Good manufacturing practices for biological products* (***11)*** should apply to vaccine filled in the final form.

Care should be taken to ensure that the materials of which the container and, if applicable, transference devices and closure are made do not adversely affect the quality of vaccine.

The manufacturers should provide the national regulatory authority with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.9 Control tests on final vaccine lot

Samples should be taken from each final vaccine lot to be tested and fulfill requirements of this section. All the tests and specifications including methods used and permitted concentrations under this section, unless otherwise specified, should be approved by the national regulatory authority.

A.9.1 Inspection of containers

Each container of each final vaccine lot should be inspected visually and those showing abnormalities should be discarded.

A.9.2 Appearance

Visual inspection of the appearance of the vaccine should be described with respect to the form and color.

A.9.3 Identity

All antigens present in final vaccine lot should be identified in each final container lot by appropriate methods. The potency test may serve as the identity test.

A.9.4 Sterility tests for bacteria and fungi

Each final lot should be tested for bacterial and fungal sterility according to the requirements in Part A, section 5.2 of *Requirements for biological substances no. 6* (***14)***, or by acceptable methods.

A.9.5 pH and osmolarity

The pH value and osmolarity of a pool of final containers should be tested.

A.9.6 Preservatives

Each final lot should be tested for the presence of preservative, if added.

A.9.7 Test for pyrogenic substances

Each final lot should be tested for pyrogenic substances. Where appropriate, tests for endotoxin should be performed. However, where there is interference in the test, e.g. because of the addition of an immunostimulant such as MPL, a test for pyrogens in rabbits should be performed, The test is conducted until consistency of production is demonstrated..

A.9.8 Adjuvant content

Each final vaccine lot should be assayed for the mineral vehicle content . Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.

Should an immunostimulant be present, each final vaccine lot should be assayed for the immunostimulant content.

A.9.9 Protein content

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

A.9.10 Degree of adsorption

The degree of adsorption (completeness of adsorption) of each antigen present in each final vaccine lot should be assessed and the limits approved by the national regulatory authority.

This test may be omitted for routine lot release upon demonstration of the product consistency.

A.9.11 Potency

The potency of each final vaccine lot should be assessed with an appropriate method in vivo or in vitro and the limits approved by the national regulatory authority. If an in vivo potency test is used, this test may be alternatively performed on the final bulk.

If an in vivo test is used, the method and the analysis of data should be approved by the national regulatory authority. The vaccine potency should be compared with that of a reference preparation and the limits of potency should be agreed with the national regulatory authority. The national regulatory authority should approve the reference preparation used.

If an in vitro test is used, an appropriate test for antigen potency should be performed on samples representative of the final vaccine lot. The test method for antigen potency could be quantitative with respect to the antigen content or relative to a reference preparation and should be appropriately validated.

Because of the diversity in the reactivity of vaccines containing HPV VLPs produced by different manufacturing techniques and to which different adjuvants have been added, it is unlikely that an International Standard for each HPV type will be suitable for the standardization of assays. Manufacturers should therefore establish a product specific reference preparation which is traceable to efficacy as demonstrated in clinical trials.

A.9.12 General safety (innocuity) test

Each final lot should be tested for unexpected toxicity (sometimes called abnormal toxicity) using a general safety (innocuity) test.

It is worth noting that this test is the only in vivo test for the vaccine product before administration to humans if an in vivo potency test is not performed.

if an in vivo potency test is performed for each final lot, it would be worthwhile to explore if the in vivo potency test can replace this test for the sake of reducing number of animals for in vivo testing although the purpose and dose regimen of the in vivo potency test are clearly different from those of the general safety test.

This test may be omitted for routine lot release once consistency of production has been well established to the satisfaction of the national regulatory authority and when good manufacturing practices are in place. Each lot, if tested, should pass a test for general safety.

A.10 Records

The requirements given in section 8 of *Good manufacturing practices for biological products* (***11)*** should apply.

A.11 Retained samples

The requirements given in section 9 of *Good manufacturing practices for biological products* (***11)*** should apply.

A.12 Labelling

The requirements given in section 7 of *Good manufacturing practices for biological products* (***11)*** should apply, with the addition of the following information.

The label on the carton, the container or the leaflet accompanying the container should state:

- that the vaccine has been prepared from recombinant yeast cells or recombinant baculovirus/insect cells or else;
- the genotype of HPV, from which L1 VLP was derived, present in the preparation;
- potency per dose;
- the number of doses, if the product is issued in a multiple-dose container;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the name and concentration of any preservative added;
- the name and concentration of any adjuvant added;
- the temperature recommended during storage and transport;
- the expiry date; and
- any special dosing schedules.

A.13 Distribution and transport

The requirements given in section 8 of *Good manufacturing practices for biological products* (***11)*** should apply.

A.14 Stability testing, storage and expiry date

A.14.1 Stability testing

Adequate stability studies form an essential part of vaccine development. The stability of the vaccine in its final form and at the recommended storage temperatures should be demonstrated to the satisfaction of the national regulatory authority on final containers from at least three lots of final product.

The formulation of vaccine antigens and adjuvant (if used) must be stable throughout its shelf-life. Acceptable limits for stability should be agreed with national authorities.

A.14.2 Storage conditions

The final container vaccine should be kept at +2 °C to +8 °C. If other storage conditions are used, they should be fully validated and approved by the national regulatory authority. The vaccine should have been shown to maintain its potency for a period equal to that between the date of release and the expiry date. During storage, liquid vaccines should not be frozen.

A.14.3 Expiry date

The expiry date should be fixed upon the approval of the national regulatory authority, and should take account of the experimental data on stability of the vaccine.

Part B. Nonclinical evaluation of recombinant HPV VLP vaccines

Nonclinical evaluation of HPV vaccines should be based on *Guidelines on nonclinical evaluation of vaccines* (***1***). This will apply to already developed vaccines when one or more additional types are added. It will also apply to new vaccines, with and without additional types compared to the vaccines available. The following specific issues should be considered in the context of the development of an HPV L1 VLP based vaccine.

B.1 Pharmacological studies

There is no adequate, relevant animal model for human papillomavirus infection, as the papillomaviruses are species-specific. The proof-of-concept for the approach to use L1 VLPs based vaccines has been demonstrated in animal protection models using “homologous” viruses, such as rabbit cottontail papillomavirus. These preclinical challenge studies in various animal models indeed demonstrated that L1 VLP are potent immunogens that induce high titers of neutralizing antibodies and protect against associated lesions. Furthermore,

transfer of serum from L1 VLP vaccinated animals provided protection in non vaccinated animals challenged with the virus.

Based on these data

- No further proof-of-concept studies need to be performed for monovalent or multivalent HPV L1 VLP vaccine.
- Neutralizing antibodies are probably the primary mediator of this protection. It seems unlikely that CMI is involved as a direct effector mechanism of protection

It is recommended that pharmacodynamic properties of an L1 VLP-based vaccine be studied through immunogenicity studies (rodents and possibly non-human primates) which should take into account:

- The evaluation and characterization of the neutralizing antibodies induced against each of the HPV L1 VLP types included in the proposed vaccine.
- In case of the inclusion of a specific adjuvant in the vaccine, the desired immune response (humoral and/or cellular, e.g. involvement of T-helper cells or induction of specific memory cells) should be supported by adequate studies in relevant species.
- The potential need to evaluate other antibody (e.g. mucosal) responses and/or cellular immune responses, to characterize the immune response more in depth.
- The generation of supportive data with respect to the relative quantitative ratio of the vaccine components.

B.2 Safety Pharmacology studies

As no effects other than on the immune system are expected with HPV vaccines based on the absence of specific toxins, safety pharmacological studies are not required.

B.3 Toxicology studies

Toxicology studies should be undertaken in accordance with the WHO guidance (***1)***. Such studies should reflect the intended clinical use of the vaccine and may include the administration of doses prior to and during gestation (***1)***. Because the target population for the HPV vaccines includes women of child-bearing age, reproductive and developmental toxicity studies are required.

In case a novel adjuvant is introduced with the HPV vaccine, the adjuvant needs to be fully characterized with adequate pharmaco/toxicological studies taking into account existing guidelines.

If a novel cell substrate is used for the production of an HPV VLP vaccine, safety aspects, in particular, any immune response elicited by residual host cell proteins,

should be investigated, including the potential for hypersensitivity reactions to occur.

Part C. Clinical evaluation of recombinant HPV VLP vaccines

This section covers:

- General recommendations for the assessment of immune responses to HPV VLP vaccines;
- Considerations for the design of studies of protective efficacy. This section briefly describes the clinical development programs for the first two vaccines to have been developed. Consideration is then given to how the protective efficacy of any future HPV VLP vaccines containing types 16 and 18 (± types 6, 11) and any HPV VLP vaccines containing HPV types not included in the first two vaccines to be developed might be assessed;
- The design of studies intended to bridge efficacy as demonstrated in sexually active young adults to other populations by means of comparisons of immune responses;
- Points to consider for the assessment of safety during clinical studies; and
- Issues that should feature in plans to monitor safety and effectiveness in the post-licensure period.

Note that:

- While this section covers some of the issues that are specific to HPV VLP vaccines G*uidelines on the clinical evaluation of vaccines*: *regulatory expectations* (***2)*** is considered applicable;
- At the time of preparing this guidance it should be noted that the first two HPV VLP vaccines were still undergoing regulatory review in many countries with only a limited number of approvals thus far; and
- There is currently no international consensus regarding how future HPV VLP 16 and 18 (± 6 and 11) vaccines should be evaluated for their protective efficacy or how the likely protective efficacy of HPV VLP vaccines that contain HPV types other than 16 and 18 (±6 and 11) might be assessed. Therefore, any manufacturers who are considering developing such vaccines are strongly recommended to seek appropriate regulatory guidance before embarking on clinical development programs. This document can only discuss some approaches that might be considered.

C.1 Immune responses to the vaccine

C.1.1 Assays

The initial assessment of immune responses to HPV VLP vaccines should be based on measurement of neutralizing antibodies in serum. Although it is not considered necessary to attempt to measure antibody responses in other milieu (e.g. cervical mucosal fluid) exploratory studies are encouraged. At the time of preparing this guidance it should be noted that there is a lack of standardization of neutralizing antibody assays although the WHO is coordinating work in this area. Therefore, careful validation of in-house methods is needed.

In-vitro neutralization tests involve measurement of the inhibition of HPV infection of cultured cells and usually employ pseudovirions carrying a marker plasmid to easily score infection. These assays detect antibodies most likely to be relevant to protection. However, they are complex, labor-intensive and not amenable to high throughput.

Competitive immunoassays utilizing neutralizing monoclonal antibodies that bind to conformational epitopes on L1 are sensitive, type specific and do not measure antibodies to denatured L1 protein. However, only a subset of the total anti-VLP antibodies are measured as binding to only one neutralizing epitope is monitored. Therefore, the results may under-represent the total protective antibody level.

HAI measures potentially protective antibodies but it is relatively insensitive and will assay only the subset of neutralizing antibodies that block cell surface binding. HAI will also measure antibodies to denatured L1 protein.

Once the neutralizing antibody response has been well characterized (see below), the sequential use of alternative assay methods, such as EIA, may be proposed. However, the routine use of EIA to assess specific antibody levels would have to be supported by a detailed analysis of the correlation between results obtained with EIA and with neutralization tests. These are simple and sensitive assays but they do not distinguish between neutralizing and non-neutralizing antibodies.

C.1.2 Characterization of the immune response

The following matters should be addressed:

- The kinetics of antibody responses (i.e. changes in antibody levels over time) to each major antigen of the vaccine should be described. As mentioned above, the focus should be on demonstrating functional antibody responses in sera. It is not considered necessary that antibody classes or subclasses are determined;

- Neutralizing antibody responses to vaccination should be compared to responses to natural infection for specific HPV types;

- Antibody responses to vaccination should be compared between individuals seronegative for each specific HPV type included in the vaccine and those already seropositive for each individual type prior to the first dose;

- Data should be provided on increments in antibody levels after each dose of vaccine to support the choice of regimen to be taken forward into confirmatory clinical studies;

- The potential for immune interference between HPV VLP vaccines and other routine vaccines that might need to be given at the same time for convenience should be investigated in order to make recommendations regarding concomitant use;

- The ability of a vaccine to elicit cross reacting neutralizing antibody should be assessed i.e. elicitation of neutralizing antibody to HPV types other than those included in the vaccine. The extent of these experiments may be limited according to existing knowledge regarding the relatedness of certain HPV types to each other;

- If additional HPV VLP types are added to an approved vaccine any effects of the extra types on immune responses to the VLPs previously included should be assessed;

- If the vaccine contains a novel adjuvant the effect of this on the immune response (humoral and/or cellular, e.g. involvement of T-helper cells or induction of specific memory cells) should be investigated;

- Induction of immune memory should be assessed by means of evaluating immune responses to additional doses of vaccine administered at planned intervals following completion of the primary series; and

- Long-term follow-up of antibody levels (e.g. up to 10 years) in vaccinated cohorts will be important. In conjunction with effectiveness data these serological data may be used to help assess whether booster doses are needed and, if so, when. Special attention should be paid to characterizing the immune status, including type-specific antibody levels, in any breakthrough cases that might occur.

At the time of preparing this guidance no immunological correlate of short-term or long-term protection (ICP) has been established for any HPV VLP type. Plans should be in place to explore data on longer-term immune responses in cohorts studied for protective efficacy and/or effectiveness in an ongoing fashion in order to attempt to identify an ICP. If an ICP were to be identified for a specific HPV type it should be noted that it might not necessarily be applicable to all other HPV types.

Currently, the lack of an ICP hinders the derivation of clinically meaningful criteria to be used in assessments of immune interference or comparisons of immune responses between populations (see also section C.3). Therefore it is recommended that such studies should evaluate both differences in seroconversion rates and geometric mean titres.

C.2 Studies of protective efficacy

C.2.1 Vaccines developed to date

Cervical cancer was not considered to be a feasible primary efficacy variable for the conduct of confirmatory studies of efficacy with HPV VLP vaccines. This is because of the long study duration that would be needed in order to obtain enough cases to make a judgment of efficacy. Therefore, the focus of the clinical development programs with respect to the evaluation of protective efficacy conferred by inclusion of HPV VLPs of types 16 and 18 in vaccines has been the prevention of CIN 2 and 3 together with AIS due to these types (***23-25)***.

Since the first two HPV VLP vaccines have been developed over approximately the same timeframe and with no licensed vaccine against HPV-related diseases available during that period it has been ethically possible to compare each of these vaccines with a placebo control group (or in some cases other unrelated vaccines have been administered to the control group).

Efficacy can only be assessed in an at-risk population. Therefore, sexually active females between the ages of approximately 15-26 years of age were enrolled into these studies.

However, it must be stressed that HPV VLP vaccines are intended to be used prophylactically. That is, to be given to individuals before they might become naturally infected with the HPV types included in the vaccines. Therefore, section C.3 considers the design of studies intended to support the extrapolation of efficacy as demonstrated in sexually active young females to children and adolescents before sexual debut by means of comparing immune responses.

In contrast, due to observed lower immune responses to HPV VLP vaccines in women older than about 26 years, studies that employ immunogenicity data to bridge efficacy from younger females are not appropriate. Therefore, ongoing studies in women older than 26 years are focusing primarily on the prevention of CIN2/3 or AIS.

Due to the above considerations for prophylactic use, some studies have allowed enrolment only of women who were seronegative and PCR negative at the screening visit for types 16 and 18 while others have allowed the inclusion of women who were already PCR and/or seropositive for types 16 and/or 18.

The primary analyses of these studies have compared the incidence of CIN2/3 and AIS in the vaccinated group with the placebo group. Although it would be more usual to consider the intent-to-treat (ITT) population as primary in placebo-controlled studies, HPV VLP vaccines were developed for prophylactic use. Therefore, the primary analysis has been based on rates of CIN2/3 and AIS associated with vaccine HPV types that have occurred in women who had no evidence of infection with the relevant vaccine types prior to vaccination and who received all three vaccine doses approximately on schedule. Thus the primary analysis is based on what may be deemed "true vaccine failures" and this is an appropriate approach.

However, in studies in which women regardless of their HPV infection or cervical disease status were allowed to be enrolled and were vaccinated and followed up, very valuable information regarding what may be expected from these vaccines may be gained from secondary and/or exploratory analyses in populations defined according to their infection status at baseline. The results so far have indicated that women already infected with one of types 16 or 18 can be protected against development of CIN2/3 or AIS associated with the other type by vaccination. Very importantly, the data available thus far indicate that HPV VLP vaccines have no therapeutic effect.

Secondary efficacy variables that have been explored in some but not all studies have included:

- High grade vulvar precancerous lesions (VIN 2/3);
- High grade vaginal precancerous lesions (VaIN 2/3);
- Low grade cervical dysplasia (CIN 1);
- Anal carcinoma;
- Persistent infection causally related to HPV types 6, 11, 16 and 18;
- Persistent infection, dysplasia and neoplasia associated with HPV types other than 16 or 18;
- Condyloma acuminata (genital warts) causally related to type 6 or 11; and
- Incident infections by HPV of types included in or not included in the vaccines. (It should be noted that although incident infection data have been explored, this is not considered to be an appropriate endpoint).

With regard to persistent infection, at the time of preparing this guidance there was no international consensus on a definition for HPV persistence based on detection of HPV DNA by restricted PCR. Various authors have proposed that the definition might be based on detection of the same HPV type in cervicovaginal samples taken 6, 12 or 18 months apart. Currently, as more data on histological endpoints and viral persistence are being obtained over a period of at least 12 months attempts are being made to further evaluate the correlation between the continued detection of an oncogenic HPV type and the development of pre-cancerous lesions and cervical cancer. Therefore, the matter of a potential

definition for viral persistence and the correlation with histological changes should be kept under close review.

With regard to the potential for any cross-protection conferred by HPV VLP vaccines against types not included in the product, it should be noted that there is currently no established definition for cross protection. Therefore, no definitive criteria exist for supporting a claim for cross-protection. As already mentioned, cross-neutralization studies with sera from vaccinees may suggest some potential for cross protection but these data cannot be used to predict efficacy against non-vaccine types. Therefore efficacy data are needed to demonstrate the potential for cross-protection. This may be explored by looking at:

- Incidences of morphological lesions (such as CIN of any grade, CIN2/3 or AIS) due to the types in question. However, numbers of cases of CIN2+ associated with HPV types other than 16 and 18 are small; and/or

- Viral persistence (see above).

With regard to the vaccine in which HPV VLPs of types 6 and 11 are also included the incidence of external genital warts (condyloma acuminata) compared to placebo has been the focus of the assessment of protective efficacy. In this case the assessment of efficacy may be made in both males and females but should be examined separately.

C.2.2 New vaccines containing HPV VLPs of types 16, 18 (± 6, 11)

The approaches to the evaluation of new vaccines containing at least types 16 and 18 might include:

- A placebo-controlled study for a new vaccine containing types 16 and 18 would not be possible once there has been widespread approval of the first two vaccines to be developed. However, not all countries will implement HPV vaccines in their routine vaccination programs. Therefore, depending on local ethical considerations, it might be possible to identify country(ies) in which HPV VLP vaccines are not being used routinely and perform a study in which the protective efficacy of the novel vaccine can be compared with an unvaccinated (i.e. standard of care) contemporaneous control group. It may be necessary to limit the duration of such a study so that the unvaccinated group is offered vaccine after a prescribed period of time. Also, a public health decision to introduce routine HPV vaccination would necessarily terminate such a study;

- Due to the concerns mentioned above, it might be preferable to perform a study in which the vaccinated cohort is resident in a country(ies) that have excellent historical data (e.g. national registry data) that could be used instead of an unvaccinated contemporaneous control group;

- Studies that compare the relative efficacy studies of the novel vaccine to a licensed vaccine containing types 16 and 18 using histological endpoints could be considered. However these studies are likely not feasible since very large numbers would need to be enrolled to provide reliable statistical analyses based on a non-inferiority study design; and

- A prospective study using a virological endpoint such as persistent HPV infection as detected in cervico-vaginal samples could compare the novel vaccine with an approved vaccine although the numbers involved would again likely have to be very large to provide reliable statistical results. In addition, if some such proposal was to be considered acceptable by regulatory authorities it would be anticipated that a commitment would have to be made to gather data on histological endpoints as a long-term follow-up commitment in the post-licensure period.

The inclusion of HPV types 6 and 11 in a novel vaccine or the addition of these types to an existing vaccine that contains types 16 and 18 could be supported by data on the incidence of condyloma acuminata in groups administered the vaccine that contains types 6 and 11 versus a vaccine containing only types 16 and 18.

C.2.3 Vaccines containing HPV VLPs of other types

Such vaccines may result from:

- Addition of HPV types (other than 6 and 11; see above) to an approved vaccine containing at least types 16 and 18; or

- De novo development of a vaccine containing other HPV VLP types (it is assumed below that this will be in addition to at least types 16 and 18).

This section is relevant to the addition of oncogenic HPV types capable of producing cancerous lesions of the cervix if left untreated. It should therefore be taken into consideration that some of these types commonly cause incident infections and are often found in association with low grade histological lesions (CIN 1) but are much less commonly found in association with CIN2/3 or AIS compared to types 16 and 18. These features seem to reflect a difference in natural histories of infections between HPV types. Due to these issues, a pre-licensure assessment based on a CIN2/3 or AIS endpoint is unlikely to be feasible.

The approaches to the evaluation of new vaccines containing additional HPV types to 16 and 18 might include:

- Comparison of rates of persistent HPV infection with each of the additional types based on detection in cervico-vaginal samples between a group that receives the novel vaccine and a group that receives an approved vaccine

containing types 16 and 18. Thus, the comparative group represents a placebo group with respect to the additional types. An exploration of the impact on low grade histological lesions could be included. Long-term follow-up (i.e. post-licensure) for impact on combined high grade histological lesions associated with the added HPV types would be an important and necessary commitment.

- If an ICP is established (for one or more HPV types (see above) it might be possible to base an initial approval on immunological responses to the added types. However, a post-licensure commitment to follow histological lesions would again be essential.

It would be important that any negative effect of adding HPV VLP types to types 16 and 18 should be assessed. This should already have been assessed serologically (see C.1) but also needs to be assessed by following up vaccinees for histological lesions and for persistent infection with types 16 and 18 in the post-licensure period.

If there are already data to suggest that the HPV VLPs of types 16 and 18 in the vaccine might confer some degree of cross protection (see above) to certain other HPV types then justification for inclusion of these other types should be provided.

C.3 Bridging efficacy by means of immunogenicity data

As discussed in section C.2, for reasons of feasibility, studies of protective efficacy have been performed in sexually active young women enrolled into large, multi-country studies. It is already clear from such studies that these vaccines should be given before individuals encounter oncogenic HPV types in order to achieve their full potential. In order to support the possibility of administering a complete primary vaccination course before sexual debut is likely to occur, immunogenicity data may be compared between cohorts of sexually active women (e.g. aged 15-26 years) who have been studied for protective efficacy and younger individuals.. Since there is a potential for these vaccines to be administered to pre-adolescent or adolescent males as well as females it would be appropriate that data on immune responses to vaccination should be obtained from and compared between genders.

It is not considered necessary that efficacy data need to be bridged by means of immunogenicity studies between healthy adult females studied for efficacy and similar individuals resident in parts of the world in which studies may not have been conducted. However, bridging studies based on immunogenicity data might be applicable for the evaluation of possible protective efficacy in immunocompromised persons, including those with HIV infection or with chronic illnesses.

The current lack of an ICP raises some difficulties regarding the assessment criteria for the demonstration of non-inferiority in bridging studies, which should be carefully justified. Since seroconversion rates to types 16, 18, 6 and 11 have been extremely high the comparison may need to focus on geometric mean titers (GMTs). In addition, it is recommended that the comparisons should be based on neutralizing antibody titres at least in a subset of the total numbers vaccinated rather than wholly basing the comparisons on data generated with EIA.

C.4 Vaccine safety

Guidelines on the clinical evaluation of vaccines: regulatory expectations (***2***) is applicable to the general evaluation of the safety of HPV VLP vaccines. This section covers only some of the issues that are specific to HPV VLP vaccines.

It is important that safety data regarding local and systemic reactogenicity are collected for adequate periods of time after each dose and that the total duration of follow-up for safety is justified. There should be plans in place to assess long-term safety by means of extended follow-up of cohorts into the post-licensure period as appropriate.

Some important issues to be addressed with regard to HPV VLP vaccines are:

- Safety should be assessed in persons who are seronegative and persons who are seropositive for one or more HPV types included in the vaccine prior to the first dose. This information has practical applications since vaccinees will not be screened for past exposure to HPV before vaccination is commenced during routine immunization programs;

- There should be adequate safety data obtained to support the potential for administration of the primary series to individuals before their sexual debut;

- The outcomes of any accidental pregnancies that occur during clinical studies should be actively sought and carefully described and assessed; and

- There should be careful monitoring of infants (e.g. growth and general health measures) who are breastfed at some time during a primary vaccination series administered to the mothers.

- If a novel cell substrate is used for the production of an HPV VLP vaccine and preclinical and/or clinical investigations have shown that an immune response is elicited to one or more residual host cell proteins then the potential implications for vaccine safety (e.g. the possibility that hypersensitivity reactions could occur) should be explored.

C.5 Post-marketing studies and surveillance

The following issues should be included in specific plans for the post-licensure assessment of HPV VLP vaccines:

- Long term follow-up of antibody status at least in selected cohorts of vaccinated persons, including adult women and representative cohorts from any population to which efficacy was bridged by means of comparison of immune responses. This will be particularly important for those who were first vaccinated before sexual debut since it is not known whether any protection afforded by vaccination will persist up to and beyond the onset of sexual activity;

- Effectiveness in terms of prevention of CIN2/3, AIS and cervical carcinoma should be assessed in the longer-term. This may be achieved by making use of existing screening programs, cancer registries, long-term follow-up of women enrolled into clinical studies that employed histological endpoints and targeted effectiveness studies in specific countries or areas where routine vaccination is introduced and there is an appropriate infrastructure in place to collect sound data. Such data, in conjunction with serological follow-up, will help to determine the need for and timing of booster doses. Ultimately, it may also be possible to identify an ICP based on accumulation of such data;

- Studies of effectiveness should also include virological assessments in order to establish whether widespread use of vaccines containing types 16 and 18 might lead to replacement of these as the predominant oncogenic HPV types in man. These data may also provide further information on the potential for types 16 and 18 to confer some degree of cross-protection against other HPV types;

- It is possible that vaccine distribution issues could lead to some individuals receiving more than one HPV VLP vaccine product to complete the primary series. This practice cannot be recommended due to the current lack of data. However, if this does occur on a large scale in a particular country/region public health authorities should take the opportunity to collect data on the outcome in terms of safety and effectiveness; and

- It is currently anticipated that vaccination with HPV VLP vaccines will not be recommended during a known pregnancy since there is no urgent need to vaccinate that would justify such use. However, inadvertent pregnancies will occur as happened during clinical studies. Detailed assessments should be made of pregnancies that occur during administration of a primary series with pro-active follow-up to detail outcomes.

Part D. Guidelines for national regulatory authorities

D.1 General

The general recommendations for control laboratories given in *Guidelines for national authorities on quality assurance for biological products* (***26)*** should apply. These guidelines specify that no new biological substance should be released until consistency of manufacturing and quality as demonstrated by a consistent release of batches has been established. The detailed production and control procedures and any significant changes in them should be discussed with and approved by the national regulatory authority. For control purposes, the national regulatory authority should obtain the working reference from manufacturers .

D.2 Release and certification

A vaccine lot should be released only if it fulfils the national requirements and/or Part A of the present Guidelines. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the national regulatory authority in support of a request for release of vaccine for use.

A statement signed by the appropriate official of the national control laboratory should be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these Guidelines. The certificate should also state the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, the date of the last satisfactory determination of antigen concentration as well as assigned expiry date on the basis of shelf life should be stated. A copy of the official national release document should be attached. The certificate should be based on the model given in Appendix 2. The purpose of the certificate is to facilitate the exchange of recombinant human papillomavirus VLP vaccines between countries.

Authors

The first draft of these Guidelines (WHO/BS/06.2050) was prepared by Dr M. Ferguson, National Institute of Biological Standards & Control, Potters Bar, United Kingdom, Ms T. Jivapaisarnpong, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, Dr R. Dobbelaer, Scientific Institute of Public Health, Bruxelles, Belgium, Dr R. Levis, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, USA, Dr S. de Andrade Nishioka, Agencia Nacional de Vigilância Sanitária, Brasilia, Brasi, Dr S. Pagliusi and Dr J. Shin, World Health Organization, Geneva, Switzerland after an informal WHO consultation held in Geneva, Switzerland (23-24 March 2006) attended by the following participants:

Dr R. Dobbelaer, Scientific Institute of Public Health, Brussels, Belgium (Chairperson); Dr M. Ferguson, National Institute of Biological Standards & Control, Potters Bar, United Kingdom (Rapporteur); Ms T. Jivapaisarnpong, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand; Dr T. Kanda, Center for Pathogen Genomics, Tokio, Japan; Dr B. Klug, European Medicines Agency, London, United Kingdom; Dr R. Levis, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, USA; Dr D. Nardelli-Haefliger, Institut de Microbiologie, Lausanne, Switzerland; Dr S. de Andrade Nishioka, Agencia Nacional de Vigilância Sanitária, Brasília, Brazil; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, London, United Kingdom; Dr J. Schiller, National Cancer Institute, Bethesda, USA; Dr J.W. van Der Laan, National Institute for Public Health and the Environment, Bilthoven, the Netherlands; Dr Y. Wang, National Institute for the Control of Pharmaceutical and Biological Products, Beijing, People's Republic of China; Dr K.-N. Zhao, Centre for Immunology and Cancer Research/University of Queensland, Queensland, Australia; Dr C. Chan, Merck Research Laboratories, Westpoint, USA; Dr M. Dennehy, The Biovac Institute, Cape Town, South Africa; Dr M. Deschamps, GlaxoSmithKline, Rixensart, Belgium; Dr M.-T. Martin, GlaxoSmithKline, Rixensart, Belgium; Dr C. Revathy, Shanta Biotechnics Pvt. Ltd, Banjara Hills, India; Dr J. Sinkule, Virionics Corporation, Scottsdale, USA; Dr R.D. Sitrin, Merck Research Laboratories, Westpoint, USA; Dr M. Suhardono, PT. Bio Farma (Persero), Bandung, Indonesia; Dr R. Biellik, Immunization Solutions, PATH - Europe, Ferney-Voltaire, France; Dr R. Krause, International Federation of Pharmaceutical Manufacturers Associations, Geneva, Switzerland; Dr K. Ardi, Regional Office for South-East Asia, World Health Organization, New Delhi, India; Dr J. P. Ruz, Pan American Health Organization, Washington DC, USA; Dr N. Dellepiane, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland; Dr S. Pagliusi, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland; Dr J. Shin, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland; Dr D. Wood, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland.

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Dr R. Dobbelaer, Scientific Institute of Public Health, Brussels, Belgium (Chairperson); Dr M. Ferguson, National Institute of Biological Standards & Control, Potters Bar, United Kingdom (Rapporteur); Ms T. Jivapaisarnpong, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand;

Dr T. Kanda, Center for Pathogen Genomics, Tokio, Japan; Dr B. Klug, European Medicines Agency, London, United Kingdom; Dr R. Levis, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, USA; Dr S. de Andrade Nishioka, Agencia Nacional de Vigilância Sanitária, Brasilia, Brasil; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, London, United Kingdom; Dr J. Schiller, National Cancer Institute, Bethesda, USA; Dr J. W. van Der Laan, National Institute for Public Health and the Environment, Bilthoven, the Netherlands; Dr Y. Wang, National Institute for the Control of Pharmaceutical and Biological Products, Beijing, People's Republic of China; Dr R. Wagner, Paul-Erhlich-Institut, Langen, Germany; Dr L. Slamet, Narcotic, Psychotropic and Addictive Substance Control, Directorate General of Food and Drug Control, Jakarta, Indonesia; Dr I. Uhnoo, Medical Products Agency, Uppsala, Sweden; Dr C. Chan, Merck Research Laboratories, Westpoint, USA; Dr. D. Jenkins, GlaxoSmithkline Biologicals, Rixensart, Belgique; Dr M. Deschamps, GlaxoSmithKline, Rixensart, Belgium; Dr M.-T. Martin, GlaxoSmithKline, Rixensart, Belgium; Dr R.D. Sitrin, Merck Research Laboratories, Westpoint, USA; Dr M. Suhardono, PT. Bio Farma (Persero), Bandung, Indonesia; Dr J. Smith, Chapel Hill University, Road Chapel Hill, North Carolina, USA; Dr R. Biellik, Immunization Solutions, PATH - Europe, Ferney-Voltaire, France; Dr S. Pagliusi, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland; Dr J. Shin, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland; Dr D. Wood, Quality, Safety and Standards, World Health Organization, Geneva, Switzerland.

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References

1. **World Health Organization**. WHO guidelines on nonclinical evaluation of vaccines. In: *WHO Expert Committee on Biological Standardization*. *Fifty-fourth report*. Geneva, World Health Organization, 2005, Annex 1 (WHO Technical Report Series, No. 927) (http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf).

2. **World Health Organization**. WHO guidelines on clinical evaluation of vaccines: regulatory expectations. In: *WHO Expert Committee on Biological Standardization*. *Fifty-second report*. Geneva, World Health Organization, 2004, Annex 1 (WHO Technical Report Series, No. 924) (http://www.who.int/biologicals/publications/trs/areas/vaccines/clinical_evaluation/035-101.pdf).

3. **Walboomers JM et al.** Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*, 1999, **189**:12-9 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10451482).

4. **International Agency for Research on Cancer**. IARC monographs on on the evaluation of carcinogenic risks to Humans: human papillomaviruses. *Vol. 90*. In preparation, (http://monographs.iarc.fr/ENG/Meetings/vol90.php).

5. **Doorbar J.** The papillomavirus life cycle. *J Clin Virol*, 2005, **32 Suppl 1**:S7-15 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15753007).

6. **Inglis S et al.** Chapter 11: HPV vaccines: Commercial Research & Development. *Vaccine*, 2006, **24 Suppl 3**:S99-S105 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16950023).

7. **World Health Organization**. Technical workshop on international collaborative studies on HPV reagents for laboratory diagnostic procedures: a progress report. Geneva, World Health Organization, 2004, (WHO/IVB/04.22) (http://www.who.int/vaccines-documents/DocsPDF04/www786.pdf).

8. **Quint WG et al.** Results of the first World Health Organization international collaborative study of detection of human papillomavirus DNA. *J Clin Microbiol*, 2006, **44**:571-9 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16455914).

9. **Ferguson M et al.** Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. *Int J Cancer*, 2006, **118**:1508-14 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16184553).

10. **Pagliusi S et al.** Chapter 23: International standard reagents for harmonization of HPV serology and DNA assays - an update. *Vaccine*, 2006, **24 Suppl 3**:S193-200 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16950007).

11. **World Health Organization**. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization*. *Forty-second report*. Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 822) (http://www.who.int/biologicals/publications/trs/areas/vaccines/gmp/WHO_TRS_822_A1.pdf).

12. **World Health Organization**. Requirements for the use of animal cells as in vitro substrates for the production of biologicals (requirements for biological substances no. 50). In: *WHO Expert Committee on Biological Standardization*. *Forty-seventh report*. Geneva, World Health Organization, 1998, Annex 1 (WHO Technical Report Series, No. 878) (http://www.who.int/biologicals/publications/trs/areas/vaccines/cells/WHO_TRS_878_A1Animalcells.pdf).

13. **World Health Organization**. Requirements for the use of animal cells as in vitro substrates for the production of biologicals (addendum 2003). In: *WHO Expert Committee on Biological Standardization*. *Fifty-fourth report*. Geneva, World Health Organization, 2005, Annex 4 (WHO Technical Report Series, No. 927) (http://www.who.int/biologicals/publications/amendment_cell_substrates_ecbs_nov_2003.pdf).

14. **World Health Organization**. General requirements for the sterility of biological substances (revised 1973). In: *WHO Expert Committee on Biological Standardization*. *Twenty-fifth report*. Geneva, World Health Organization, 1973, Annex 4 (WHO Technical Report Series, No. 530) (http://www.who.int/biologicals/publications/trs/areas/vaccines/sterility/WHO_TRS_530_A4.pdf).

15. **World Health Organization**. General requirements for the sterility of biological substances (requirements for biological substances no. 6, revised 1973, amendment 1995). In: *WHO Expert Committee on Biological Standardization*. *Forty-sixth report*. Geneva, World Health Organization, 1998, Annex 3 (WHO Technical Report Series, No. 872) (http://www.who.int/biologicals/publications/trs/areas/vaccines/sterility/WHO_TRS_872_A3.pdf).

16. **World Health Organization**. Recommendations for the production and control of poliomyelitis vaccine (oral). In: *WHO Expert Committee on Biological Standardization*. *Fiftieth report*. Geneva, World Health Organization, 2002, Annex 1 (WHO Technical Report Series, No. 904) (http://www.who.int/biologicals/publications/trs/areas/vaccines/polio/WHO_TRS_904_A1polio_oral.pdf).

17. **World Health Organization**. Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products. In: *WHO Expert Committee on Biological Standardization*. Geneva, World Health Organization, 2003, (http://www.who.int/biologicals/publications/en/whotse2003.pdf, accessed 20 June 2006).

18. **World Health Organization**. Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (requirements for biological substances no. 27). In: *WHO Expert Committee on Biological Standardization*. *Forty-third report*. Geneva, World Health Organization, 1994, Annex 2 (WHO Technical Report Series, No. 840) (http://www.who.int/bloodproducts/publications/WHO_TRS_840_A2.pdf).

19. **World Health Organization**. Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology. In: *WHO Expert Committee on Biological Standardization*. *Forty-first report*. Geneva, World Health Organization, 1991, Annex 3 (WHO Technical Report Series, No. 814) (http://www.who.int/biologicals/publications/trs/areas/vaccines/rdna/WHO_TRS_814_A3.pdf).

20. **International Conference on Harmonization**. Quality of biotechnological products: analysis of the expression construct in cells used for production of recombinant DNA derived protein products. Q5B. Step 4 version. 1995, ICH Harmonized Tripartite Guideline (http://www.ich.org/LOB/media/MEDIA426.pdf).

21. **World Health Organization**. Requirements for measles, mumps and rubella vaccines and combined vaccines (live) (requirements for biological substance no. 47). In: *WHO Expert Committee on Biological Standardization*. *Forty-third report*. Geneva, World Health Organization, 1994, Annex 3 (WHO Technical Report Series, No. 840) (http://www.who.int/biologicals/publications/trs/areas/en/index.html).

22. **International Conference on Harmonization**. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. Q5A (R1). Step 4 version. 1999, ICH Harmonized Tripartite Guideline (http://www.ich.org/LOB/media/MEDIA425.pdf).

23. **Pagliusi SR, Teresa Aguado M.** Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine*, 2004, **23**:569-578 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15630792).

24. **United States Food and Drug Administration**. Summary minutes VRBPAC. 2001, (http://www.fda.gov/ohrms/dockets/ac/01/minutes/3805m1.pdf#search=%22vrbpac%202001%20hpv%22 accessed 15 Sept 2006).

25. **United States Food and Drug Administration**. VRBPAC background document: Gardasil™ HPV quadrivalent vaccine. May 18, 2006 VRBPAC meeting. Bethesda, Maryland. 2006, (http://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4222B3.pdf accessed 15 Sept 2006).

26. **World Health Organization**. Guidelines for national authorities on quality assurance for biological products. In: *WHO Expert Committee on Biological Standardization*. *Forty-second report*. Geneva, World Health Organization, 1992, Annex 2 (WHO Technical Report Series No. 822) (http://www.who.int/biologicals/publications/trs/areas/en/index.html).

Appendix 1

Model summary protocol for manufacturing and control of recombinant human papillomavirus virus-like particle vaccine

The following protocol is intended for providing general guidance, and indicates the information that should be provided as a minimum by the manufacturer to the national regulatory authority. The protocol must be accompanied by a lot release certificate from the licensing authority which may or may not be the country of manufacturing origin. Information and tests may be added or deleted as required by the national regulatory authority of the importing country, if applicable.

It is thus possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the license and with the relevant WHO guidelines of a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it must also be accompanied by a lot release certificate from the national regulatory authority of the country in which the vaccine was produced stating that the product meets national requirements as well as Part A of this WHO guidelines document.

1. Summary information on the finished product (final lot)

International name: _____

Trade name: _____

Batch number(s):

Finished product (final lot): _____

Final bulk: _____

Type of container: _____

Total number of containers in this batch: _____

Number of doses per container: _____

Composition (antigen concentration) / volume of single human dose: _____

_____

Date of expiry: _____

Storage temperature: _____

Product license (marketing authorization) number: _____

Name and address of manufacturer: _____

Name and address of product license holder if different: _____

2. Production information

Batch number of each monovalent bulk: _____
Site of manufacture of each monovalent bulk: _____
Date of manufacture of each monovalent bulk: _____
Site of manufacture of final bulk: _____
Date of manufacture of final bulk: _____
Site of manufacture of finished product : _____
Date of manufacture of finished product: _____

A genealogy of the lot numbers of all vaccine components used in the formulation of the final product will be informative.

The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine

3. Starting materials

The information requested below is to be presented on each submission. Full details on Master and working seed-lots and cell banks upon first submission only and whenever a change has been introduced.

3.1 Cell banks

Source of HPV antigen (expression system) _____
Master cell bank (MCB) lot number & preparation date: _____
Population doubling level (PDL) of MCB _____
Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the marketing authorisation: _____
Manufacturer's working cell bank (MWCB) lot number & preparation date: _____
Population doubling level (PDL) of MWCB _____
Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the marketing authorisation: _____
Production cell lot number: _____

Identification of cell substrate

Method:

Specification:

Date:

Result:

Nature and concentration of antibiotics or selecting agent (s) used in production cell culture maintenance medium:

Identification and source of starting materials used in preparing production cells including excipients and preservatives (particularly any materials of human or animal origin e.g. albumin; serum):

3.2. Virus seed lots if a recombinant baculovirus expression vector is used

Virus strain and reference number used to prepare the licensed HPV vaccine:

Master seed lot number & preparation date:

Number of passages between two seeds mentioned above:

Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the marketing authorization:

Working seed lot number & preparation date:

Passage level from Master seed lot:

Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the marketing authorization:

Sufficient detail should be provided for any additional 'sub' working seed lots including the passage level from the master seed and the length and conditions of storage if any.

Each seed lot should be tested for the followings

Identity

Method:

Specification: _____
Date: _____
Result: _____

Bacteria and fungi
Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

Mycoplasmas, spiroplasma, entomoplasma and mesoplasma _____
Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

Adventitious agents
Method: _____
Specification: _____
Date: _____
Result: _____

Mycobacterium spp. (if applicable)
Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

Recombinant baculovirus concentration
Method: _____
Specification: _____
Date: _____
Result: _____

3.3 Control cell cultures if mammalian or insect cells are used for production

Provide information on control cells corresponding to each single harvest.

Ratio or proportion of control to production cell cultures: _____

Volume of control cells: _____

Period of observation of cultures: _____

Percentage rejected for non-specific reasons: _____

Result: _____

Karyotype :

- Method: _____
- Probe : _____
- Reference cells : _____
- Date test on: _____
- Date test off: _____
- Result: _____

Identity test by DNA finger printing (if applicable)

- Method: _____
- Probe: _____
- Reference cells: _____
- Restriction enzymes: _____
- Date test on: _____
- Date test off: _____
- Result: _____

Haemadsorbing viruses

- Type(s) of RBC: _____
- Storage time and temperature of RBC: _____
- Incubation time and temperature of RBC: _____
- % cultures tested: _____
- Date test on: _____
- Date test off: _____
- Result: _____

Tests on supernatant fluids for other adventitious agents (if relevant)

Date of sampling from production cell cultures: _____

Type of simian cells: _____
- Quantity of sample inoculated: _____
- Incubation temperature: _____
- Date test on: _____
- Date test off: _____
- % of viable culture at the end _____
- Result: _____

Type of human cells: _____
- Quantity of sample inoculated: _____
- Incubation temperature: _____
- Date test on: _____
- Date test off: _____
- % of viable culture at the end: _____
- Result: _____

Type(s) of other diploid cells: _____
- Quantity of sample inoculated: _____
- Incubation temperature: _____
- Date test on: _____
- Date test off: _____
- % of viable culture at the end: _____
- Result: _____

Bacteria and fungi _____
- Method _____
- Media: _____
- Volume inoculated: _____
- Date test on: _____
- Date test off: _____
- Result: _____

Mycoplasmas _____
- Method: _____
- Media: _____
- Volume inoculated: _____
- Date test on: _____

Date test off: _____
Result: _____

4. Single harvests (or pools)

Batch Number(s): _____
Date of inoculation: _____
Date of harvesting: _____
Volume(s) of fermentation paste, storage temperature, storage time and approved storage period: _____

Culture purity or Sterility for bacteria and fungi

Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

Identity of host strain

Method: _____
Specification: _____
Date: _____
Result: _____

Consistency of yield (e.g. infectivity of replicating vector virus and/or HPV antigen concentration, if applicable)

Method: _____
Reference preparation : _____
Specification: _____
Date: _____
Result: _____

In addition, the following tests if mammalian cells or insect cells are used

Adventitious agents

Method: _____
Specification: _____
Date: _____

Result: _____

Mycoplasmas

Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

5. Purified monovalent antigen bulk

Batch number(s) of purified bulk: _____
Date(s) of purification(s): _____
Volume(s), storage temperature, storage time and approved storage period: _____

Identity (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Composition (protein, lipid, polysaccharide, if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Protein purity (add PAGE photographs)

Method: _____
Specification: _____
Date: _____
Result: _____

Protein content

Method: _____
Specification: _____
Date: _____

Result: _____

Antigen content (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Ratio of antigen : protein content (if applicable)

Specification: _____
Result: _____

Bacteria and fungi

Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

Percent intact L1 monomer:

Method: _____
Specification: _____
Date: _____
Result: _____

VLP size and structure

Report on this is needed until production consistency is demonstrated

Method: _____
Specification: _____
Date: _____
Result: _____

Potential hazards e.g. residual chemical(s) (if relevant)

Method: _____
Specification: _____
Date: _____
Result: _____

Residual DNA (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Albumin content

(if mammalian or insect cells and animal serum are used for production)

Method: _____
Specification: _____
Date: _____
Result: _____

Viral clearance

This is performed during vaccine manufacturing development and/or process validation and is not intended for batch release. See section A.5.1.12

Method: _____
Specification: _____
Date: _____
Result: _____

6. Adsorbed monovalent antigen bulk

Batch number(s) of adsorbed monovalent antigen bulk: _____
Adsorption date: _____
Batch number(s) of all components used during adjuvant adsorption: _____
Volume, storage temperature, storage time and approved storage period: _____

Bacteria and fungi

Method: _____
Media: _____
Volume inoculated: _____
Date test on: _____
Date test off: _____
Result: _____

Bacterial endotoxins

Method: _____
Specification: _____
Date: _____
Result: _____

Identity
Method: _____
Specification: _____
Date: _____
Result: _____

Adjuvant or mineral vehicle concentration (if relevant)
Method: _____
Specification: _____
Date: _____
Result: _____

Degree of adsorption (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

pH
Method: _____
Specification: _____
Date: _____
Result: _____

Antigen content (in vitro assay)
Method: _____
Batch number of reference vaccine and assigned potency: _____
Specification: _____
Date: _____
Result: _____

In vivo assay (where applicable)
Species, strain, sex and weight specifications: _____

Dates of vaccination, bleeding: _____

Date of assay: _____

Batch number of reference vaccine and assigned potency: _____

Vaccine doses (dilutions) and number of animals responding at each dose: _____

ED_{50} of reference and test vaccine: _____

Potency of test vaccine vs. reference vaccine with 95% fiducial limits of mean: _____

Validity criteria: _____

VLP size distribution (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Freezing point (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

7. Final vaccine bulk

Batch number: _____

Date of manufacture: _____

Batch numbers and volumes of adsorbed bulk vaccines used for the formulation of the final bulk vaccine: _____

Batch number(s) and volume(s) of bulk alum diluent: _____

Volume, storage temperature, storage time and approved storage period: _____

Bacteria and fungi

Method: _____

Media: _____

Volume inoculated: _____

Date test on: _____

Date test off: _____

Result: _____

Adjuvants

Method: _____
Specification: _____
Date: _____
Result: _____

Degree of adsorption (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Preservatives (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Potency

If an in vitro assay of each type is used

Method: _____
Batch number of reference vaccine and assigned potency: _____
Specification: _____
Date: _____
Result: _____

If an *in vivo* assay is used

Species, strain, sex and weight specifications: _____
Dates of vaccination, bleeding: _____
Date of assay of each type: _____
Batch number of reference vaccine and assigned potency: _____
Vaccine doses (dilutions) and number of animals responding at each dose for each type: _____
ED50 of reference and test vaccine for each type: _____

Potency of test vaccine vs. reference vaccine for each type with 95% fiducial limits of mean: _____

Validity criteria for each type: _____

8. Final vaccine lot

Batch number: _____

Date of filling: _____

Type of container: _____

Filling volume: _____

Number of containers after inspection: _____

Appearance

Method: _____

Specification: _____

Date: _____

Result: _____

Identity (each type)

Method: _____

Specification: _____

Date: _____

Result: _____

Bacteria and fungi

Method _____

Media: _____

Volume inoculated: _____

Date test on: _____

Date test off: _____

Result: _____

pH

Method: _____

Specification: _____

Date: _____

Result: _____

Osmolarity

Method: _____

Specification: _____

Date: _____
Result: _____

Preservatives (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Pyrogenic substances
Method: _____
Specification: _____
Date: _____
Result: _____

Adjuvant content
Method: _____
Specification: _____
Date: _____
Result: _____

Protein content (or calculated value)
Method: _____
Specification: _____
Date: _____
Result: _____

Degree of adsorption of each type (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Potency:
In vitro assay of each type
Method: _____
Batch number of reference vaccine and assigned potency: _____
Specification: _____
Date: _____

Result: _____

If an *in vivo* assay is used (may be performed at final bulk stage)

Species, strain, sex and weight specifications: _____

Dates of vaccination, bleeding: _____

Date of assay of each type: _____

Batch number of reference vaccine and assigned potency: _____

Vaccine doses (dilutions) and number of animals responding at each dose for each type: _____

ED50 of reference and test vaccine for each type: _____

Potency of test vaccine vs. reference vaccine for each type with 95% fiducial limits of mean: _____

Validity criteria for each type: _____

Date of start of period of validity: _____

General safety (unless deletion authorised)

Method: _____

Specification: _____

Date: _____

Result: _____

Freezing point (if applicable)

Method: _____

Specification: _____

Date: _____

Result: _____

Appendix 2

Model certificate for the release of recombinant human papillomavirus virus-like particle vaccine

This certificate is to be provided by the national regulatory authority of the country where the vaccines have been manufactured, upon request by the manufacturer

Certificate Nº _____

LOT RELEASE CERTIFICATE

The following lot(s) of recombinant human papillomavirus virus-like particle vaccine produced by _____[1] in _____[2], whose numbers appear on the labels of the final containers, meet all national requirements[3] and Part A[4] of the *WHO guidelines to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines* (_____)[5], and comply with *Good Manufacturing Practices for Pharmaceutical Products: Main Principles*[6] and *Good Manufacturing Practices for Biological Products*[7].

As a minimum, this certificate is based on examination of the summary protocol of manufacturing and control.

Final Lot No.	No. of released human doses in this final lot	Expiry date
_____	_____	_____

The Director of the National Regulatory Authority (or Authority as appropriate):

Name (Typed) _____
Signature _____
Date _____

[1] Name of manufacturer

[2] Country of origin

[3] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national regulatory authority

[4] With the exception of provisions on distribution and shipping, which the national regulatory authority may not be in a position to assess.

[5] WHO Technical Report Series, No. ___, YYYY, Annex __.

[6] WHO Technical Report Series, No. 908, 2003, Annex 4.

[7] WHO Technical Report Series, No. 822, 1992, Annex 1.