Annex 1

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, held on 23–24 March and 28–30 August 2006, at which scientific experts, regulatory professionals and other stakeholders met to develop guidelines for prophylactic vaccines against human papillomavirus (HPV). These guidelines are intended to provide background and guidance to national regulatory authorities 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. Guidance specific to the nonclinical evaluation of recombinant HPV vaccines is provided in part B and on their clinical evaluation in part C. 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 rather than recommendations. Guidelines allow greater flexibility than recommendations with respect to expected future developments in the field. This guidance is based on the experience with the products developed so far, as described below, and may need to be updated in response to 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 six early proteins responsible for virus replication and two late proteins, L1 and L2, which are the viral structural proteins. L1 is the major structural protein and the L1 proteins associate to form pentameric structures called capsomers. Mature virus particles comprise 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 occur in the stratified squamous epithelia of skin and mucous membranes, with virus spread occurring through 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 labelled high risk for oncogenicity. Other HPV types, such as types 6 and 11 associated with genital warts, are considered low risk for oncogenicity.

The majority of HPV infections with both high and low risk types are asymptomatic, self-limiting and resolve spontaneously, presumably due to the
host immune response. In some instances, persistent infection with 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 is usually
about 10 years or longer. High-risk HPV types can be detected in virtually all
women with 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 understood. However, environmental and
physiological cofactors may increase the risk for cancer development in people
with persistent infection.

The International Agency for Research on Cancer (IARC) currently
defines 13 high-risk HPV types that are associated with cancers in humans
(4). Distribution and prevalence of these HPV types in patients with cancer 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 that of 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, although rare disease that manifests as recurrent, rapidly growing
benign laryngeal tumours that require frequent excision to prevent airway
obstruction. HPV 6 and 11 are responsible for over 90% of genital warts and cases
of RRP, and 9–12% 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. The 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 experimental infection with
a high dose of homologous virus. HPV VLPs are highly immunogenic in mice and 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 provide protection 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 vaccination with VLP. 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 labelled neutralizing monoclonal antibodies, haemagglutination inhibition (HAI), and in vitro neutralization.

The development of these guidelines has been driven by the experience acquired with the two vaccines developed thus far. These vaccines are both made up of recombinant protein L1 VLPs and they contain adjuvant 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:

- a bivalent vaccine comprising 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
- a tetravalent vaccine comprising 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 adjuvant containing amorphous aluminium hydroxyphosphate sulfate.

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, nonclinical 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:

- 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.
- A novel adjuvant with which experience has not previously been gained 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.
- L1 protein in its native form is not glycosylated. For the two currently available 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.
- Disassembly and reassembly of the L1 capsomers may contribute to purification of the product and lead to more stable VLPs.
- Purified L1 VLP preparations will need to be characterized biochemically and immunologically, to determine L1 concentration, purity and assembly state.
- The currently available HPV vaccines are manufactured in single-dose forms 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, their use should be time-restricted as is the case for reconstituted vaccines such as Bacillus Calmette-Guérin (BCG) and measles-containing vaccines. If a preservative were to be added, its effect on antigenicity and immunogenicity must be assessed and it must be known not to have a negative impact as has been observed with thiomersal (6).

It is crucial that the nonclinical studies demonstrate immunogenicity and the production of neutralizing antibodies.

For the clinical assessment of HPV VLP vaccines there are several critical considerations:
Since 90% of HPV-related cancers affect the cervix, the efficacy of the vaccines developed so far has been studied in sexually active women.

In order to obtain maximal benefit from these vaccines, the primary target population for immunization should be young adolescents before the onset of sexual activity. Although the attack rate for HPV is high in the 5–10 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.

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.

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 end-point 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.

Once licensed, evaluation of the long-term effectiveness of these vaccines should be integrated with current screening programmes 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 parentheses by the genotype specificity and the name of the 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 meet 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 (from now on 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 these Guidelines were prepared. However, reference reagents for use in the laboratory evaluation of the biological effects following administration of vaccine to humans, such as antibody titres and detection of viral DNA, 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 the terms used in these guidelines. They may have different meanings in other contexts.

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

**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.

**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.

**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 or below for yeast, and in liquid nitrogen for insect or mammalian cell lines).

**End of production cells:** a cell suspension containing the cells harvested at the end of culture or fermentation.

**Fermentation cell paste:** a suspension of cells harvested at the end of the yeast fermentation stored frozen (−60 °C or below).

**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.

**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.

**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.

**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.

**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.

**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 or below 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.

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

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

**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.

**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.

**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 homogeneous pool of multiple single harvests of the intended HPV antigens of one virus type, collected into a single vessel before clarification.

**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
or below 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 the vaccine product.

A.2 General manufacturing recommendations

The general manufacturing requirements contained in *Good manufacturing practices for biological products* (1) 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 production of recombinant 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.
- 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 an example of a process key operating parameter is column loading. The process validation antigen batches should show compliance with the pre-established 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 analysis of the terminal amino acid sequence.

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 standards of 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. An 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 MCB and WCB. 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 examined for tumorigenicity in an animal test system and for the presence of retroviruses and arthropod-borne viruses (12).

The MCB is made in sufficient quantities and stored in a secure environment and is used as the source material to make the manufacturer’s WCB. In normal practice an 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 which is distributed into ampoules and preserved cryogenically to form the WCB.

The manufacturer’s WCB 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 freedom 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 done on 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, ovine or cervine 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 paroviruses, as applicable. 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 the 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 they are 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 at 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

MCBs and WCBs should be tested for the absence of adventitious bacteria, fungi and mycoplasmas according to Part A, section 5.2 of the 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 a 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 from 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 occur without showing 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 national regulatory authority.

Viruses of invertebrates include ascoviruses, baculoviruses, birnaviruses, dicistroviruses, iridoviruses, metavirus, nodaviruses, paroviruses, polydnaviruses, poxviruses, pseudoviruses, reoviruses and tetraviruses. However, their relevance to the species of origin of the insect cells used in cell banks and production was largely unknown 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 transmissible spongiform encephalopathy (TSE) agents if bovine serum is used.

Full characterization may be performed on either the MCB or on the WCB (12).

A.3.3.3 Other cell substrates
Tests on the MCB and WCB are performed in accordance with the 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 manufacturer’s recombinant baculovirus working seed lots. Either the virus master seed lots or the virus working seed lots should be fully characterized and tested extensively for adventitious agents, and should be approved by the national regulatory authority.

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

The manufacturer’s 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 a defined number of passages from the recombinant baculovirus master seed lot by a method and a passage level from the original virus seed approved by the national regulatory authority. 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 the 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 have any 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 a special emphasis on potential insect-borne human pathogens (e.g. arboviruses). The specificity and sensitivity of assays should be defined and approved by the national regulatory authority.

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 details of the tests see the 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 national regulatory authority.

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

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

A.3.4.1.6 Recombinant baculovirus concentration

Each recombinant baculovirus seed lot should 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 the fermentor or bioreactor with the intention to feed cells or to induce or 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 specific characteristics of the recombinant baculovirus strain. 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 °C or below.

A.4.2.1 Tests on control cell cultures

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 attributable 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 under essentially similar conditions to those used for 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 than 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 °C or below.

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

For the test to be valid, at least 80% of the control cells should still survive 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 °C.

In some countries, the national regulatory authority requires that additional tests for haemadsorbing viruses are to be performed using red blood cells of other species including 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 °C, and again after incubation for a further 30 minutes at 20–25 °C. The test with monkey red blood cells should be read once more after an additional period of incubation for 30 minutes at 34–37 °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 survive at 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 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 cm² per ml of pooled fluid. At least one bottle of each kind of cell culture 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 survive at 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 include, but are 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 the 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 allow it 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 a 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 and before 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 °C 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. Alternatively, the identity can 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, has been withheld from the corresponding final bulk.

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 the 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, 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 A.2 and approved by the national regulatory authority. Any agent added in the purification process, such as Benzonase, should be documented.

The monovalent antigen purified bulk can be stored under conditions shown by the manufacturer to allow it 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 subjected 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 the Requirements for biological substances no. 6 (14), or by a method approved by the national regulatory authority. Alternatively, this test can be performed on the related adsorbed monovalent antigen bulks if the purified bulk is not stored prior to adsorption.

A.5.1.6  Percentage of intact L1 monomer
The percentage of intact L1 protein may be assessed by suitable methods until production consistency is demonstrated.

A.5.1.7  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 national regulatory authority.

A.5.1.8  Tests for agents used during purification or other phases of manufacture
A test should be done to detect 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.5.1.9  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 suitably sensitive methods. The level of host cell DNA should not exceed the maximum level cited in the 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 residuals being tested for from the monovalent antigen purified bulks.
A.5.1.10  **Albumin content**

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

A.5.1.11  **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 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q5A guidelines (22). This testing is performed during vaccine manufacturing development and/or process validation and is not intended for use with 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 an aluminium salt. In that case, the mineral vehicle and the 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 allow it 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 adsorbed 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 the *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 bulk 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 using 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 the 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, this 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 at the concentration used. Until the bulk is filled into containers, the final bulk suspension should be stored under conditions shown by the manufacturer to allow it 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 the concentrations permitted 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.

An example of a suitable test for immunostimulants such as MPL is gas chromatography.

Tests for adjuvants may be conducted on each final vaccine lot 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 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, the 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 for the following tests. They should be tested and fulfil the requirements discussed below in 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**
The appearance of the vaccine should be described with respect to the form and colour observed in a visual inspection.

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, for example 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 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 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. Alternatively, if an in vivo potency test is used, this test may be 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 method...
of testing 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 to be done 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 whether the in vivo potency test can replace this test thus reducing the number of animals required 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
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 the shelf-life of the vaccine. Acceptable limits for stability should be agreed with national regulatory 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 the Guidelines on nonclinical evaluation of vaccines (1). These guidelines will apply to already developed vaccines when one or more additional types are added. They will also apply to new vaccines, with and without additional types compared to those included in the vaccines already available. The following specific issues should be considered in the context of the development of an HPV L1 VLP-based vaccine.

8.1 Pharmacological studies

There is no adequate, relevant animal model for HPV infection, as the papillomaviruses are species-specific. The proof-of-concept for the use of 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 have indeed demonstrated that L1 VLPs are potent immunogens that induce high titres of neutralizing antibodies and protect against associated lesions. Furthermore, transfer of serum from animals vaccinated with L1 VLP provided protection in nonvaccinated 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 the pharmacodynamic properties of an L1 VLP-based vaccine be assessed through immunogenicity studies (in rodents and possibly in 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 the 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 suitable 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 in more depth;
the generation of supportive data with respect to the relative 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 pregnancy (1). Because the target population for the HPV vaccines includes women of child-bearing age, reproductive and developmental toxicity studies are required.

In the case that a novel adjuvant is introduced with the HPV vaccine, the adjuvant needs to be fully characterized with adequate pharmacological and 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 programmes 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 (with or without types 6 and 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, the *Guidelines on the clinical evaluation of vaccines: regulatory expectations* (2) are considered applicable.
- At the time of preparing this guidance, the first two HPV VLP vaccines were still undergoing regulatory review in many countries and had so far only received a limited number of approvals.
- There is currently no international consensus regarding how future HPV VLP 16 and 18 (with or without 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 (with or without 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 programmes. This document can only discuss some of the 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 body fluids (e.g. cervical mucosal fluid), exploratory studies are encouraged. It should be noted that at the time of preparing this guidance there was a lack of standardization of neutralizing antibody assays although 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 allow infection to be scored easily. These assays detect antibodies most likely to be relevant to protection. However, they are complex and labour-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 level of protective antibodies.
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 those obtained 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 for antibody classes or subclasses to be 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 individuals who are already seropositive for each individual type before the first dose.
- Data should be provided on increments in antibody levels after each dose of vaccine to support the choice of regimen that will be used in subsequent 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 the sake of 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 depending on the 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 evaluating immune responses to additional doses of vaccine administered at planned intervals following completion of the primary series.

Long-term follow-up of antibody levels (e.g. up to 10 years) in vaccinated cohorts will be important. In conjunction with data on effectiveness 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) had been established for any HPV VLP type. Plans should be in place to explore data on longer term immune responses ongoing in the cohorts studied for protective efficacy and/or effectiveness 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 the ICP identified 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 to obtain enough cases to make a judgement of efficacy. Therefore, the focus of the clinical development programmes 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 period and no licensed vaccine against HPV-related diseases has been available during that period, it has been ethically possible to compare each of these vaccines with a placebo-treated 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 women between approximately 15 and 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, they are 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, current studies in women older than 26 years are focusing primarily on the prevention of CIN2/3 or AIS.

In light of 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-positive 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 that in the placebo-treated group. Although it would be more usual to consider the intention-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 showed no evidence of infection with the relevant vaccine types before 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 whether or not they had an HPV infection or cervical disease, were allowed to be enrolled and were vaccinated and followed up, 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 either type 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 with HPV of types included in or not included in the vaccines. (It should be noted that although data on incident infection have been explored, this is not considered to be an appropriate end-point.)

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 end-points 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.

In relation 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, the numbers of cases of CIN2+ associated with HPV types other than 16 and 18 are small); and/or
- viral persistence (see above).
In studying the vaccine in which HPV VLPs of types 6 and 11 are also included, the incidence of external genital warts (condyloma acuminata) in vaccinated individuals compared to those treated with a 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 the results should be examined separately.

C.2.2 New vaccines containing HPV VLPs of types 16, 18 (with or without 6 and 11)

The approaches to the evaluation of new vaccines containing at least types 16 and 18 might take into account the following:

- A placebo-controlled study of a new vaccine containing types 16 and 18 would no longer be possible once there had been widespread approval of the first two vaccines to be developed. However, not all countries will implement HPV vaccination as part of their routine vaccination programmes. Therefore, depending on local ethical considerations, it might be possible to identify a country or countries 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 can be offered the vaccine after a prescribed period of time. Also, a public health decision to introduce routine HPV vaccination would necessarily end such a study.

- In light of the concerns mentioned above, it might be preferable to perform a study in which the vaccinated cohort is resident in a country or countries 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 of the novel vaccine to that of a licensed vaccine containing types 16 and 18 using histological end-points could be considered. However these studies are unlikely to be feasible since very large numbers of participants would need to be enrolled to allow reliable statistical analyses based on a non-inferiority study design.

- A prospective study using a virological end-point such as persistent HPV infection as detected in cervico-vaginal samples could compare the novel vaccine with an approved vaccine, although the numbers of participants involved would again probably have to be very large to
provide reliable statistical results. In addition, if such a proposal was to be considered acceptable by the regulatory authorities it would be anticipated that a commitment would have to be made to gather data on histological end-points as a long-term follow-up obligation 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 comparing the incidence of condyloma acuminata in groups administered the vaccine that contains types 6 and 11 with data on recipients of 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 than types 16 and 18. These features seem to reflect a difference in natural histories of infections between HPV types. Thus a pre-licensure assessment based on a CIN2/3 or AIS end-point 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 comparison 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 to assess any negative effect of adding HPV VLP types to types 16 and 18. 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) against 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, to achieve their full potential, these vaccines should be given before individuals encounter oncogenic HPV types. 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 for data on immune responses to vaccination to be obtained from both males and females and compared between them.

It is not considered necessary for efficacy data to be bridged by means of immunogenicity studies between healthy women 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 people, 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 titres (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**

The *Guidelines on the clinical evaluation of vaccines: regulatory expectations* (2) are 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 as follows:

- Safety should be assessed in people who are seronegative and people who are seropositive for one or more of the HPV types included in the vaccine before 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 programmes.
- Adequate safety data should be obtained to support the potential for administration of the primary series to individuals before their sexual debut.
- Information on the outcomes of any accidental pregnancies that occur during clinical studies should be actively sought and carefully documented and assessed.
- There should be careful monitoring of infants (e.g. for 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 **Postmarketing studies and surveillance**

The following issues should be addressed 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 people, 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 programmes, cancer registries, long-term follow-up of women enrolled into clinical studies that employed histological end-points 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 humans. 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 owing to the current lack of data. However, if this does occur on a large scale in a particular country or region, public health authorities should take the opportunity to collect data on the outcome in terms of safety and effectiveness.

■ It is currently anticipated that vaccination with HPV VLP vaccines will not be recommended in women known to be pregnant 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 proactive follow-up to document the outcomes.
Part D. Guidelines for national regulatory authorities

D.1 General
The general recommendations for control laboratories given in the 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 expiry date assigned 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.

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References


Appendix 1

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

The following protocol is intended to provide 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 licence and with the relevant WHO guidelines on 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 licence (marketing authorization) number: ______________________
Name and address of manufacturer: ________________________________
Name and address of product licence 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 are requested 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 and 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 authorization: _________________________
Manufacturer's working cell bank (MWCB) lot number and 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 authorization: _________________________
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 or 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 and 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 and 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: ____________________________

Each seed lot should be tested for the following:

Identity
Method: __________________________________________________________
Specification: ______________________________________________________
Date: _____________________________________________________________
Result: ____________________________________________________________

Bacteria and fungi
Method: __________________________________________________________
Media: ____________________________________________________________

---

a 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.
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 red blood cell (RBC): ________________________________
Storage time and temperature of RBC: ________________________________
Incubation time and temperature of RBC: ________________________________
Percentage 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: ________________________________
Percentage of viable culture at the end: ________________________________
Result: ________________________________
Type of human cells:
- Quantity of sample inoculated: ____________________________
- Incubation temperature: ________________________________
- Date test on: __________________________________________
- Date test off: __________________________________________
- Percentage 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: __________________________________________
- Percentage 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, information on the following tests should be provided 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: ________________________________
Percentage 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: 

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: _______________________________________________________
<table>
<thead>
<tr>
<th>Test Type</th>
<th>Method</th>
<th>Specification</th>
<th>Date</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>Volume inoculated</td>
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<td>Date test on</td>
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<td>Date test off</td>
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<td>pH</td>
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<td>Osmolarity</td>
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<tr>
<td>Preservatives (if applicable)</td>
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<td>Pyrogenic substances</td>
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<td>Protein content (or calculated value)</td>
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<td>Result</td>
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</tbody>
</table>
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:  
ED$_{50}$ 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 authorized)
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 requirements3 and Part A4 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 principles6 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.

<table>
<thead>
<tr>
<th>Final lot no.</th>
<th>No. of released human doses in this final lot</th>
<th>Expiry date</th>
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</thead>
<tbody>
<tr>
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</table>

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.