EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
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Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines

Replacement of: TRS 962, Annex 1

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Adopted by the Sixty-sixth Meeting of the World Health Organization Expert Committee on Biological Standardization, 12 to 16 October 2015. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.
Recommendations and guidelines published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Recommendations be made only on condition that modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. The parts of each section printed in small type are comments or examples for additional guidance intended for manufacturers and NRAs, which may benefit from those details.
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Introduction

WHO’s guidelines to assure the quality, safety and efficacy of recombinant human papillomavirus (HPV) virus-like particle (VLP) vaccines were first published in 2006, as Annex 1 of the WHO Technical Report Series, No. 962 (1). Those guidelines were based largely on experience gained from clinical trials undertaken on the first two licensed HPV vaccines.

Issues that have prompted this revision include the substantial amount of data accumulated during vaccine implementation, the development of prophylactic vaccines with extended valency and the use of other production methods. In addition, the increasing availability and routine use of HPV VLP vaccines composed of L1 capsid protein and containing at least types 16 and 18 have important implications for trial designs and endpoints for clinical evaluation of new prophylactic HPV vaccines.

A series of meetings was convened by WHO to review the scientific evidence for initiating the revision of Annex 1 of the WHO Technical Report Series, No. 962. These meetings were attended by experts from around the world who were involved in the research, manufacture, licensing/authorization, control-testing and release of HPV vaccines and included academia, national regulatory authorities (NRAs)/national control laboratories (NCLs), industry, and representatives from the WHO Global HPV LabNet (an initiative that worked towards international standardization of HPV testing during 2006–2011). These experts reviewed new HPV vaccines under development and the scientific basis and evidence for accepting alternative endpoints for evaluating the clinical efficacy of candidate HPV vaccines. The first meeting held on 27 February 2013 considered issues relating to the development and evaluation of clinical endpoints for trials of new HPV vaccines and other issues to be addressed in the revision of the guidelines. At a meeting held at the WHO International Agency for Research on Cancer (IARC), Lyon, France, on 23–24 September 2013, the Working Group chiefly discussed whether it might be appropriate to consider using a virological endpoint, rather than a disease endpoint such as cervical intra-epithelial neoplasia grade 2 or worse (CIN2+), as the primary endpoint for future clinical efficacy trials, and the circumstances under which immuno-bridging trials might be sufficient for licensure (2, 3). A third meeting was held at WHO headquarters on 19–20 November 2013 to review and
discuss the outcomes of the IARC scientific meeting on appropriate clinical endpoints, to review vaccines currently in the development pipeline, and to assess regulatory and laboratory needs for licensing the vaccines (4).

Major issues addressed in this revision include updates on:
- general considerations and other sections to reflect the up-to-date development of HPV vaccines;
- the section on international standards and reference preparations;
- terminology;
- Part A, in order to include bacteria as a cell substrate;
- Part B, in order to include a new subsection highlighting test articles needed for nonclinical evaluation and to elaborate regulatory requirements for both proof-of-concept pharmacology studies and toxicological testing;
- Part C, in order to reflect appropriate and feasible endpoints in future trials;
- appendices.

Additional changes have been made to bring the document into line with other WHO recommendations published since the last revision.

**Scope**

These WHO recommendations provide guidance to NRAs and manufacturers on the manufacturing process, and on nonclinical and clinical aspects of recombinant HPV VLP vaccines to assure the quality, safety and efficacy of the vaccines.

The scope of the present document encompasses recombinant HPV VLP vaccines for prophylactic use which contain the L1 capsid protein of one or more HPV types.

The document does not cover vaccines targeted to L2 capsid proteins as antigens, as appropriate serological assays have not yet been standardized and clinical vaccine trials have not started. Non-VLP vaccines (e.g. other forms of subunit vaccines, vectored vaccines and L1 capsomers) and investigational therapeutic HPV vaccines which are at an early stage of development, are also not included. However, some aspects discussed may be relevant and may be taken into consideration during vaccine development.
This document should be read in conjunction with other relevant WHO guidelines such as those on nonclinical (5) and clinical evaluation (6) of vaccines. Other WHO guidelines, such as those for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7) and nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (8), should also be considered.

**General considerations**

HPV is not a single virus; rather, it includes a group of closely related small, non-enveloped deoxyribonucleic acid (DNA) viruses in the *Papillomaviridae* family. The circular, double-stranded viral genome is approximately 8 kb in length. The genome encodes for six early protein-coding sequences responsible for virus replication and two late proteins, L1 and L2, which are the viral structural capsid proteins. L1 is the major structural protein. L1 proteins associate to form pentameric structures called capsomers (9). 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 (10). L2 is not required for particle formation, but plays a role in encapsidating the genome. HPV infection, replication and particle maturation occur in the stratified squamous epithelia of skin and mucous membranes (11).

Over 190 different types of HPV have been identified and molecularly characterized (12). These HPVs cause a variety of epithelial diseases in humans, ranging from benign warts to cancers (including of the cervix, vagina, vulva, penis, anus and oropharynx). HPV types associated with the development of cervical cancer are labelled as high risk for oncogenicity.\(^1\) Other HPV types, such as types 6 and 11 which are associated with genital warts, are considered as low risk for oncogenicity.

The majority of HPV infections with both high-risk and low-risk types are asymptomatic, self-limiting and resolve spontaneously due to the host immune response. In rare instances, HPV infection persists. If persistent infection with high-risk types is not detected and treated, progression to invasive carcinoma may occur at the site of infection. The interval between

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\(^1\) The terms oncogenic/oncogenicity and carcinogenic/carcinogenicity are both used in scholarly articles on human papillomaviruses. The terms oncogenic/oncogenicity is used in this document.
acquisition of HPV infection and malignant (invasive) progression is generally at least 10 years. As high-risk HPV types are detected in virtually all cervical cancers, it is scientifically accepted that the persistent viral infection is necessary though not sufficient for the development of cancer (13, 14). The basis for progression to invasive carcinoma is not well understood. Environmental and physiological cofactors such as high parity, hormonal contraceptives and smoking may increase the risk for cancer development in people with persistent infection (15–17). Nevertheless, individuals without identified risk factors who have persistent infection can also develop cervical cancer.

IARC currently defines 12 high-risk HPV types that are associated with cancers in humans – i.e. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Group 1) – and an additional type as probably carcinogenic – i.e. HPV type 68 (Group 2A) (14). HPV type 66 was formerly classified as oncogenic but recent findings have significantly weakened the evidence. Additional data on rare HPV types currently considered possibly oncogenic to humans (Group 2B) suggests that some, but not all, could be upgraded (18). Distribution and prevalence of these HPV types in patients with cancer are generally consistent around the world. Two of the high-risk HPV types, 16 and 18, account for approximately 70% of all cervical cancers globally (14). Most anal cancers are also associated with persistent HPV infection, and HPV type 16 represents an even higher fraction (90%) of HPV-positive cancers of the anus than in cervical cancer (approximately 50%). In addition, these high-risk HPV types are associated with a significant fraction of cancers of the vagina, vulva, penis and oropharynx. The incidence of cervical cancer is substantially higher than that of all other HPV-related cancers; cervical cancer is the second most common cancer among women aged 15–44 years.

Low-risk HPV types cause genital warts, recurrent respiratory papillomatosis (RRP), and low-grade cervical dysplasia. Genital warts affect both males and females. Data on the worldwide burden of genital warts is not available, but in developed countries the epidemiology is similar to other sexually transmitted infections, peaking in young ages (15–24 years) (19). While not malignant, these lesions are associated with physical and psychological morbidity and may be 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 types 6 and 11 are responsible for over
90% of genital warts and cases of RRP, as well as 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 in standard cell culture. Subsequent studies showed that L1 protein produced in heterologous expression systems, such as yeast or insect cells, self-assembles into VLPs that are morphologically similar to authentic HPV virions but contain no viral DNA. In animal studies, VLPs were shown to protect against experimental infection with a high dose of homologous virus (20, 21). 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. In animal studies, immunization with denatured VLPs did not produce neutralizing antibodies and did not give protection from experimental virus challenge, indicating that neutralizing epitopes are conformation-dependent.

Protection in animals has been demonstrated through passive transfer of antibodies in serum (20–22). Neutralizing antibodies are believed to be the primary mediator of this protection. Enhanced regression of established HPV lesions, which requires cell-mediated immunity (CMI), was not observed after VLP vaccination. Therefore, it seems unlikely that CMI is substantially involved as a direct effector mechanism of protection (23). The specific assays that have been developed to evaluate the humoral immune response to HPV include: VLP-based enzyme immunoassay (EIA), competitive immunoassay with labelled neutralizing monoclonal antibodies and pseudovirion-based neutralization assays (24).

The revision of these WHO recommendations (formerly guidelines) has been driven by the experience acquired with the first two HPV L1 VLP vaccines that have been licensed in many countries and with the development of an extended version of one of the two to include additional HPV types. These vaccines are made up of recombinant protein L1 VLPs and contain adjuvant to enhance immunogenicity of the L1 VLPs. The vaccines are delivered via
intramuscular injection. 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 vaccines are:

- A bivalent vaccine comprising HPV types 16 and 18 VLPs self-assembled from L1 protein expressed and purified from insect cells infected with a recombinant baculovirus: the vaccine is formulated with a novel adjuvant, AS04, which contains aluminium hydroxide and monophosphoryl lipid A (MPL), and each dose delivers 20 µg of each VLP.

- A quadrivalent vaccine comprising HPV types 6, 11, 16 and 18 VLPs self-assembled from L1 protein expressed and purified from Saccharomyces cerevisiae (baker’s yeast) containing L1 expression plasmids: the vaccine is formulated with amorphous aluminium hydroxyphosphate sulfate adjuvant, and each dose delivers 20 µg of HPV types 6 and 18 VLPs and 40 µg of HPV types 11 and 16 VLPs.

- An extended version of the quadrivalent vaccine that contains five additional oncogenic HPV types (types 31, 33, 45, 52 and 58): the vaccine is manufactured as for the quadrivalent vaccine and contains the same adjuvant.

The bivalent vaccine expressed from recombinant baculovirus in insect cells was the first vaccine to be developed in this host expression system. Other novel expression systems, such as Escherichia coli or Pichia yeast, may be introduced. Testing of novel cell substrates may have some unique requirements. Each VLP type in a multivalent vaccine should be produced and purified separately and then mixed together for the final formulation. L1 protein in its native form is not glycosylated (25). The currently available vaccines do not utilize expression systems that glycosylate the VLP, and glycosylation does not appear to be important. Other expression systems without post-translation glycosylation, such as the E. coli expression system, could also be used.

L1 VLPs may include other recombinant proteins (e.g. L2) that serve as a stabilizer rather than as an antigen (26). Conformational epitopes of L1 VLPs are required for eliciting neutralizing antibody response in the host. Disassembly of the L1 VLPs into capsomers and reassembly of the L1 capsomers back into VLPs may contribute to purification of some types of the HPV VLPs and lead to more stable VLPs.
Purified L1 VLP preparations will need to be characterized biochemically and immunologically to determine L1 concentration, purity, post-translational modification and assembly state. L1 VLPs incorporate nucleic acids of their producer cells to varying degrees, and so the amount of residual host cellular DNA incorporated needs to be determined. Removal of host cellular DNA may be required if the amount exceeds the limits set for the specific production system by NRAs.

Regulatory approvals for the bivalent and quadrivalent vaccines were based on double-blind efficacy studies in females aged 16–26 years using unvaccinated control groups – i.e. groups that received placebo or another vaccine with no potential to protect against HPV. The primary endpoints for these Phase III clinical trials were based on histological detection of precancerous epithelial changes attributable to specific HPV types as a surrogate for risk of progression to malignant lesions (cervical intraepithelial neoplasia [CIN] grades 2 or 3 [CIN2–3] and adenocarcinoma in situ [AIS]). Secondary endpoints included 6- or 12-month type-specific persistence of HPV detection. Primary efficacy was demonstrated in women who were not previously exposed to the vaccine types. Immunogenicity and safety were established in younger males and females (ages 9–15 years), and this information was used to bridge efficacy claims to this population. On the basis of demonstrated safety and efficacy against cervical cancer precursors, WHO’s Strategic Advisory Group of Experts (SAGE) on Immunization recommended that the primary target population should be girls within the age range of 9 or 10 years through 13 years – i.e. before the age of initiation of sexual activity and exposure to HPV (11).

The initial product licenses were for 3-dose schedules (0, 1 or 2 months, 6 months). Subsequently the European Medicines Agency (EMA) approved a 2-dose schedule (0, 5–13 months) for the bivalent vaccine for females aged 9–14 years based on non-inferiority of the immune response compared to the standard 3-dose schedule in females aged 15–25 years, and a 2-dose schedule (0, 6 months) for the quadrivalent vaccine for females aged 9–13 years compared to the standard 3-dose schedule in females aged 16–26 years. For both the bivalent and quadrivalent HPV vaccines, SAGE recommended a 2-dose schedule with a 6-month interval between doses for females younger than 15 years. Those who are ≥15 years at the time of the second dose are also adequately covered by 2 doses (11).
The extended version of the quadrivalent vaccine includes five additional HPV types (31, 33, 45, 52 and 58). All nine HPV VLPs are prepared from L1 protein expressed and purified from *Saccharomyces cerevisiae* containing L1 expression plasmids. The licensure of this 9-valent vaccine was based on demonstration of efficacy (using a composite histological endpoint) against the additional HPV types and immunological bridging to clinical efficacy data for the four HPV types in the quadrivalent vaccine.

The currently available HPV vaccines are manufactured in single-dose or 2-dose vials or prefilled syringes without the addition of preservative. In future, the availability of multi-dose vaccine vials should 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-Guerin (BCG) and measles-containing vaccines (27). If a preservative were to be added, its effect on antigenicity and immunogenicity must be assessed to ensure that the preservative does not affect immune response. Thiomersal, an organo-mercury compound that was a widely used preservative in vaccines, was found to destroy the major neutralizing epitope of HPV type 16 L1 VLPs when added to the vaccine formulation (28).

Current vaccine formulations use intramuscular administration. Other routes of administration – such as nasal, cutaneous or oral – may be investigated to facilitate vaccination by avoiding the use of needles. It is crucial that nonclinical studies demonstrate immunogenicity and the production of neutralizing antibodies for any new products and any new routes of administration.

**Terminology**

The definitions given below apply to the terms used in these recommendations. They may have different meanings in other contexts.

*Adjuvant:* a substance or a combination of substances that are used in conjunction with a vaccine antigen to enhance (e.g. increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine. This may also be called a mineral vehicle or immunostimulant.
**Adsorbed monovalent antigen bulk**: a batch of purified monovalent antigen bulk adsorbed on adjuvant. Different batches of adsorbed monovalent antigen bulks may be pooled before collection into a single vessel.

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

**Adventitious agents**: contaminating microorganisms that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses, that have been unintentionally introduced into the manufacturing process.

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

The individual containers (e.g. ampoules, vials) should be representative of the pool of cells from which they are taken and should be frozen on the same day by following the same procedure and by using the same equipment and reagents.

**Cell seed**: a quantity of vials containing well-characterized cells derived from a single tissue or cell of human or animal origin stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which would be used for the production of a master cell bank.

**Cell substrate**: cells used to manufacture a biological product.

**Comparator vaccine**: a licensed vaccine with established efficacy or with traceability to a vaccine with established efficacy that is tested in parallel with a candidate vaccine and serves as an active control in nonclinical or clinical testing.

**Composite endpoints**: combine two or more single endpoints in one outcome to demonstrate overall treatment effects. Subjects who have experienced any of the events specified in the single endpoints are considered to have experienced the composite endpoint (29).
**Control cell culture:** cells amplified from the working cell bank and split from those intended for use in production immediately prior to intentional viral infection (e.g. with baculovirus expression vector) and grown in parallel for the purpose of serving as test material.

**Expression construct:** is defined as the expression vector containing the coding sequence of the recombinant protein (30).

**Expression system:** the host cell with its expression construct and the cell culture process that is capable of expressing protein encoded by the expression construct. Expression systems can be bacterial-based, baculovirus-insect cell-based, mammalian-based or yeast-based.

**Final lot:** a collection of sealed final containers of formulated vaccine that is homogeneous with respect to the risk of contamination during the filling process. A filling lot must therefore have been filled from a single vessel of final bulk or prepared in one working session.

**Final bulk:** the formulated vaccine present in a 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. If prepared from one or more monovalent antigen bulks, mixing should result in a uniform preparation to ensure that the final containers are homogenous.

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

**Immune correlate of protection (ICP):** an immune response parameter of a type (e.g. serum antibodies) and amount above which an individual is most probably protected. For many vaccines, functional antibodies above a threshold geometric mean titres (GMTs) or concentrations (GMCs) serve as an immune correlate of protection.
**Immunobridging studies**: studies intended to support the extrapolation of efficacy from one formulation, population or dose regimen to another based on comparison of relevant immune responses.

**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 from insect cells.

**L1 virus-like particle (VLP)**: a non-infectious, non-replicating, non-enveloped, icosahedral capsid particle that resembles native virions but does not contain viral DNA. The regular arrays of L1 pentameric capsomers retain conformational epitopes.

**Master cell bank (MCB)**: a quantity of well-characterized cells of animal or other origin, derived from a cell seed at a specific population doubling level (PDL) or passage level, dispensed into multiple containers, cryopreserved, and stored frozen under defined conditions, (typically −60 °C or below for yeast or bacteria, and in liquid nitrogen for insect or mammalian cell lines). The master cell bank is prepared from a single homogeneously mixed pool of cells. In some cases, such as genetically engineered cells, this may be prepared from a selected cell clone established under defined conditions. The MCB is used to derive all working cell banks for the anticipated lifetime of the vaccine product.

**Parental cells**: cells that are manipulated to give rise to a cell substrate.

  Manipulation may be simply the expansion of a primary cell culture to provide early passage cells, or a more complex activity such as developing a transfected clone. Both processes would provide a cell seed. The parental cells may refer to any stage prior to the preparation of the cell seed.

**Production cell culture**: a collection of cell cultures used for the production of HPV L1 antigen that have been prepared together from one or more containers of the working cell bank.

**Purified monovalent antigen bulk**: a batch of purified antigen of a single HPV type. Different batches of purified monovalent antigen bulks may be pooled into a single vessel.
Recombinant baculovirus master seed lot: a quantity of recombinant baculovirus of uniform composition derived from an original baculovirus expression 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 recombinant baculovirus 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 concentrated cell suspension or supernatant 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 proceeding with purification.

Working cell bank (WCB): a quantity of well-characterized cells of animal or other origin, derived from the master cell bank at a specific PDL or passage level, dispensed into multiple containers and stored under defined culture conditions (typically -60 °C or below for yeast or bacteria, and in liquid nitrogen for insect or mammalian cell lines). The working cell bank is prepared from a single homogeneously mixed pool of cells. One or more of the WCB containers are used for each production culture. Multiple WCBs may be made and used during the lifetime of the vaccine product.

Part A. Manufacturing recommendations

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 type specificity and the name of the recombinant protein (e.g. type 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 that contains purified VLPs composed of the recombinant major capsid proteins (L1) of one or more HPV types. The VLPs may be formulated with a suitable adjuvant. Such vaccines are for prophylactic use.

A.1.3 International reference preparations
International Standards for HPV types 16 and HPV 18 antibodies are available. These materials may be used in immunoassays utilizing VLP binding assays and pseudovirion neutralization tests of adequate sensitivity \((31, 32)\).

International Standards for HPV types 16 and 18 DNA are also available. These standards are suitable for calibration of in-house or working standards for the amplification and detection of HPV types 16 and 18 DNA \((33)\).

The reference materials listed above are available from the National Institute for Biological Standards and Control, Potters Bar, United Kingdom.

The WHO catalogue of international biological standards should be consulted for the latest list of appropriate WHO International Standards and reference materials \((34)\). The *Human papillomavirus laboratory manual* \((24)\) provides guidance on the preparation and use of secondary standards calibrated against International Standards for HPV DNA and antibodies.

International standards and reference reagents for the control of HPV VLP vaccine antigen content and potency are not available. Therefore, product-specific reference preparations may be used.

A.2 General manufacturing recommendations
The general manufacturing requirements contained in the WHO’s *Good manufacturing practices for pharmaceutical products: main principles* (35) and *Good manufacturing practices for biological products* (36), should apply to the design, establishment, operation, control and maintenance 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 microorganisms.
- Quality control procedures should be in place to ensure segregation of different HPV L1 VLP types during bulk manufacturing steps, as well as segregation from other products manufactured in the same facility. Sufficient cleaning validation and product changeover data should be available to evaluate procedures. The antigen manufacturing process should be validated to demonstrate production consistency. Typically, three consecutive lots per HPV type are required.

### 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) or mass spectrometry. The SDS-PAGE bands should be identified by sensitive staining techniques and where possible by specific antibodies, or mass spectrometry could be used 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 sequences.

Since it is known that conformational epitopes are essential for efficacy, it is necessary that the morphological characteristics of the VLPs and degree of aggregation should be determined. VLP characterization may be done by additional appropriate methods.

### 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 (7, 37). Only cells that have been approved and registered with the NRA should be used to produce HPV L1 VLP vaccines. The NRA should be responsible for approving the cell bank or seed lot. An appropriate history of the cell bank should be provided. With regard to cell cultures, the maximum number of passages (or population doublings) allowable from the MCB, through the WCB, and through the production in cells should be approved by the NRA.

**A.3.1.1 Recombinant yeast and bacteria cells**

The characteristics of the parental cells and the recombinant production strain (parental cell transformed with the recombinant expression construct) should be fully described and information should be given on the testing for 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. 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. Some techniques (e.g. deep sequencing) allow for the entire construct to be examined, while others (e.g. restriction enzyme analysis) allow for assessment of segments (30, 38). The molecular and physiological measures used to promote and control the expression of the cloned gene in the host cell should be described in detail (38).

The nucleotide sequence of the gene insert and the adjacent segments of the vector and restriction-enzyme mapping of the vector containing the gene insert should be provided as required by the NRA.

Cells must be maintained in a frozen state that allows for 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 recombinant (modified) host and vector are maintained and clearly identifiable. Cell banks should be identified and fully characterized by means of appropriate tests.

Data – e.g. plasmid restriction enzyme mapping, nutritional requirements or antibiotic resistance, (if applicable) – that demonstrate the genetic stability of the expression system during passage of the recombinant WCB up to or beyond the passage level used for production should be provided to and approved by the NRA. Any instability of the expression
system occurring in the seed culture or after a production-scale run should be documented. Stability studies should also be performed to confirm cell viability after retrieval from storage, maintenance of the expression system, etc. These studies may be performed as part of the routine use in production or may include samples taken specifically for this purpose.

A.3.1.1.1 Tests on recombinant yeast and bacteria MCB and WCB

MCBs and WCBs should be tested for the absence of bacterial and fungal contamination by appropriate tests, as specified in Part A, sections 5.2 (39) of WHO’s General requirements for the sterility of biological substances, or by a method approved by the NRA, to demonstrate that only the bacteria or yeast production strain is present and that the MCB and WCB are not contaminated with other bacteria or fungi.

A.3.1.2 Insect cells

If insect cells are used, cell substrates and cell banks should conform with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7), as appropriate to insect cells, and should be approved by the NRA.

The MCB is made in sufficient quantities and stored in a secure environment, and is used as the source material for making 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 NRA, at which point the cells are combined to give a single pool which is distributed into ampoules and preserved cryogenically to form the WCB. Working cell banks of insect cells may be used for recombinant baculovirus seed lot production and antigen expression.

A.3.1.2.1 Tests on insect cell MCB and WCB

Tests on the MCB and WCB should be performed in accordance with WHO’s Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7). It is important to show that the cell banks are free from bacteria, fungi, mycoplasmas, mycobacterium spp, and adventitious agents relevant to the species that may be present in raw materials used in its
derivation. For insect cell lines, special emphasis is put on potential insect-borne human pathogens (e.g. arboviruses).

Insect viruses have not been well characterized compared with other potential adventitious agents, and there is therefore less information about them – and specifically their infectivity, replicative life cycles and pathogenicity, if any. It should be borne in mind that infection of insect cells with some insect viruses may occur without showing cytopathic effect. Tests may include specific nucleic acid amplification tests (NAT) such as polymerase chain reaction (PCR) and other non-specific tests, such as electron microscopy and co-cultivation. The specificity and sensitivity of assays should be determined by the manufacturer and approved by the NRA.

Full characterization may be performed on either the MCB or on the WCB with more limited testing on the other, depending on the chosen strategy for testing (see reference 7 for further explanation). Scientific advice on the testing strategy should be sought from the NRA.

**A.3.1.3 Recombinant mammalian cells**

If mammalian cells are used, the cell substrates and cell banks should conform with WHO’s *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks* (7) and the *Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology* (38), and should be approved by the NRA.

**A.3.2 Recombinant baculovirus master and working seeds for antigen production**

The recombinant baculovirus expression vector contains the coding sequence of the HPV protein antigen. 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 on the construction, the genetics and structure of the baculovirus expression vector(s).

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.

Only recombinant baculovirus seed lots that are approved by the NRA should be used. The recombinant baculovirus master seed lot should be made in sufficient quantities to last the lifetime of the vaccine product and should be stored in a secure environment, preferably in two geographically separate locations. The master seed lot is used as the source material for making 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, while the other may be subjected to more limited testing. The testing strategy and seed lots should be approved by the NRA.

The manufacturer’s recombinant baculovirus working seed lot is used in the production of inoculum intermediates and single antigen harvests and is prepared from the master recombinant baculovirus seed lot. It is recommended that a large lot of recombinant baculovirus working seed should 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 NRA. Once the acceptable passage level of the working seed lot is established, it may not be changed in making future lots of working seed without approval from the NRA.

**A.3.2.1 Tests on recombinant baculovirus master and working seed lots**

The expression construct should be analysed using NAT in conjunction with other tests performed on the purified recombinant protein for assuring the quality and consistency of the expressed HPV L1 antigens. The genetic stability and stability of expression 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 (30, 38).

**A.3.2.1.1 Identity**
Each baculovirus master and working seed lot should be identified by the HPV type of the inserted gene by an appropriate method such as PCR. The tests should be approved by the NRA.

A.3.2.1.2 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 (39) and 5.3 (40) of the WHO General requirements for the sterility of biological substances or by a method approved by the NRA. In addition, the recombinant baculovirus seed lot should be tested for insect mollicutes (mycoplasma) such as spiroplasma, entomoplasma and mesoplasma.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and agreement from the NRA (7).

A.3.2.1.3 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 indicator cells, neutralization of baculovirus is necessary. Antisera used for this purpose should be free from antibodies that may neutralize adventitious viruses, and should preferably be generated by immunization of specific-pathogen-free animals with an antigen made from a source, other than the production cell line, which has itself been tested for freedom from adventitious agents. The inoculated indicator cells should be examined microscopically for cytopathic changes. At the end of the examination period, the cells should also be tested for haemadsorbing viruses (see A.4.2.1.1). It should be noted that infection of indicator cells by insect viruses may not reveal any cytopathic effect. Additional tests such as PCR, electron microscopy and co-cultivation may therefore be performed. 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 determined by the manufacturer and approved by the NRA.
In general, recombinant baculovirus seeds should be assessed for absence of adventitious agents that may have been introduced during their production, including those that may be present in the source materials used at each of the production stages of the master and working virus seed lots. 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 WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7).

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: degenerate NAT for whole virus families, with analysis of the amplicons by hybridization, sequencing or mass spectrometry; NAT with random primers followed by analysis of the amplicons on large oligonucleotide micro-arrays of conserved viral sequencing or digital subtraction of expressed sequences; and high throughput or deep sequencing. These methods may be used to supplement existing methods or as alternative methods to in vivo and/or in vitro tests after appropriate validation and approval by the NRA (7).

A.3.2.1.4 Test for mycobacterium species (spp)
Each recombinant baculovirus seed lot should be tested for mycobacterium spp. The test method and specifications should be approved by the NRA.

A.3.2.1.5 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.3.2.1.6 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 NRA.

A.3.3 Cell culture medium
If serum is used for the propagation of cells, it should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas – as specified in the requirements given in Part A, sections 5.2 (39) and 5.3 (40) of the WHO General requirements for the sterility of biological substances – and freedom from adventitious viruses.

Detailed guidelines for detecting bovine viruses in serum for establishing MCB and WCB are given in Appendix 1 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7) and should be applied as appropriate. The guidelines for detecting bovine viruses in serum for establishing the cell banks may also be applicable to production cell cultures. As an additional monitor of quality, sera may be examined for endotoxin. Gamma-irradiation may be used to inactivate potential contaminant viruses, recognizing that some viruses are relatively resistant to gamma-irradiation.

Whatever the process used, the validation study has to determine the consistency and effectiveness of the process while maintaining serum performance. The use of non-inactivated serum should be justified and is not advised without strong justification. The non-inactivated serum must meet the same criteria as the inactivated serum when tested for sterility and absence of mycoplasma and viral contaminants.

The source(s) of animal components used in culture medium should be approved by the NRA. These components should comply with the current WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (41).

Bovine or porcine trypsin used for preparing cell cultures should be tested and found free of bacteria, fungi, mycoplasmas and adventitious viruses, as appropriate. The methods used to ensure this should be approved by the NRA. The source(s) of trypsin of bovine origin, if used, should be approved by the NRA and should comply with the current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (41).

In some countries, irradiation is used to inactivate potential contaminant viruses. If irradiation is used, it is important to ensure that a reproducible
dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough to allow the biological properties of the reagents to be retained but also high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process (7). The irradiation method should be validated and approved by the NRA.

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free from risk of contamination and should be subject to the usual considerations for any reagent of biological origin (7).

Human serum should not be used. If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. As a minimum, it should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (42). In addition, human albumin and materials of animal origin should comply with the current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (41).

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 lot is acceptable to the NRA.

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 NRA may be added.

A.4 Control of HPV VLP production

A.4.1 Control of HPV VLP production up to single antigen harvest in yeast and bacterial expression systems

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 NRA.
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 NRA. No antibiotics should be added at any stage of manufacturing unless approved by the NRA.

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

Cell cultures are expanded to an appropriate scale and are then inoculated with recombinant baculovirus at a defined multiplicity of infection (MOI). 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 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 approved by the NRA.

A single antigen harvest is obtained within a defined time period post-inoculation. Several single antigen harvests may be pooled. If multiple single antigen harvests are pooled, each single antigen 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 manufacture. Samples of single harvest pools should be taken for testing and should be stored at a temperature of −60 °C or below.

A.4.2.1 Tests on control cell cultures

When the insect cell suspension is grown to the scale needed for production, but prior to infection with the recombinant baculovirus, 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 NRA.

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 conditions that are essentially similar to those used for the production cultures, with the agreement of the NRA. For insect cells, the above incubation time may not apply because of the specificities of cells cultivated in suspension but it should be not less than the time of collection of the single antigen harvest. At the end of the examination period, fluids collected from the control cell culture from each single antigen 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 antigen harvests prepared from these cultures should not be used for HPV VLP production.

For the test to be valid, at least 80% of the control cells should still survive by the end of the test period and any losses should be due to nonspecific or accidental reasons.

**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 NRA 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/or chickens (or other avian species).

All haemadsorption 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.

If a test with monkey red blood cells is performed, readings should also be taken after a final 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 with spent control cell culture fluid. For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period.

A.4.2.1.2 Tests for other adventitious agents
At the end of the observation period, a sample of the pooled fluid and/or cell lysate from each group of control cell cultures should be tested for adventitious agents. For this purpose, 10 ml of each pool should be tested in the same cells as those used for the production of virus, but not the same batch of cells as those used for the production of vaccine. 10-ml samples of each pool should also be tested in human cells and in a simian kidney cell line.

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

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

For the tests to be valid, not more than 20% of the culture cells should have been discarded for nonspecific accidental reasons by the end of the test period.

Some NRAs require that these cells should be tested for the presence of haemadsorbing viruses at the end of the observation period.

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 NRA. 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, PCR).
A.4.3 Control of HPV VLP production up to single antigen harvest in mammalian cells
If applicable, tests on control cells for identity and adventitious agents should be performed in accordance with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7).

A.4.4 Control of single antigen harvests
A.4.4.1 Storage and intermediate hold times
Prior to and during the purification process, the cell suspension or supernatant fluid should be maintained under conditions shown by the manufacturer to allow them to retain the desired biological activity. Hold times should be approved by the NRA.

A.4.4.2 Tests on single antigen harvest or single harvest pool
If appropriate, tests may be conducted on a single antigen harvest or on a pool of single antigen harvests. The protocol should be approved by the NRA.

A.4.4.2.1 Sampling
Samples required for the testing of single antigen harvests or single harvest pools should be taken immediately on harvesting and before further processing. If the tests for sterility and adventitious agents, as described in Part A.4.4.2.2 and Part A.4.4.2.4, 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 for bacteria, fungi and mycoplasmas
Each single antigen harvest or single harvest pool should be shown to be free from bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2, of the WHO General requirements for the sterility of biological substances (39).

Harvests from bacterial expression systems could be positive for bacterial contamination. Therefore, an alternative method such as the microbial limits test might be appropriate for addressing culture purity. Such testing should be approved by the NRA.
In addition to sterility tests for bacteria and fungi, each single antigen harvest or single harvest pool should be shown to be free from mycoplasmal contamination by appropriate tests as specified in Part A, section 5.3, of the WHO *General requirements for the sterility of biological substances* (40) if insect or mammalian cells are used in production, or by a method approved by the NRA.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and agreement from the NRA (7).

**A.4.4.2.3 Test for identity of HPV types**

Each single antigen harvest or single harvest 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 NRA. 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**

Each single antigen harvest or single harvest 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 or the mammalian cell substrate. These cell cultures should include, as a minimum, a monkey kidney cell line and a human cell line. Antisera used for the purpose of neutralizing the recombinant baculovirus should be free from antibodies that may neutralize adventitious viruses, and should preferably be generated by immunization of specific-pathogen-free animals with an antigen made from a source, other than the production cell line, which has itself been tested for freedom from adventitious agents. The inoculated indicator cells should be examined microscopically for cytopathic changes. At the end of the examination period, the cells should also be tested for haemadsorbing viruses (see A.4.2.1.1).

Additional testing for specific adventitious viruses may be performed, for instance using PCR amplification techniques.
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, and should be approved by the NRA. The maximum number of harvests that may be pooled should also be approved by the NRA. Adequate purification may require several purification steps based on different biophysical and/or biochemical principles and may involve disassembly and re-assembly of VLPs. The entire process (sequence of process steps) used for the purification of the VLPs should be appropriately validated, as described in section A.2, and should be approved by the NRA. Any reagents added during the purification processes, such as DNase, should be documented.

The purified monovalent antigen 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 NRA.

A.5.1 Tests on the purified monovalent antigen bulk

Purified monovalent antigen 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 purified monovalent antigen bulk, unless otherwise specified, should be validated by the manufacturer and approved by the NRA.

A.5.1.1 Identity

Each purified monovalent antigen bulk should be identified as the appropriate HPV antigen type by a method suitable for distinguishing between HPV types (e.g. an immunological assay). The test for antigen content may also serve as the identity test.

A.5.1.2 Purity

The degree of purity of each purified monovalent antigen bulk, and levels of residual host cell protein, 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 SDS-PAGE under reducing denaturing conditions. The protein bands within the gel should be identified by sensitive staining techniques. The protein in each band should be quantified by densitometric analysis at an appropriate level of sensitivity, in order to measure the degree of purity.
Host cell protein detection by expression specific and sensitive EIA method may be developed.

A.5.1.3 Protein content
Each purified monovalent antigen bulk should be tested for the total protein content using a suitable method.

Alternatively, the total protein content may be calculated from a measurement of an earlier process intermediate.

A.5.1.4 Antigen content
The antigen content should be measured on the purified monovalent antigen bulk or the adsorbed monovalent antigen bulk (see A.6.3.7) by an appropriate method which is type-specific.

The ratio of antigen content to protein content may be calculated and monitored for each purified monovalent antigen bulk.

International standards and reference reagents for the control of HPV VLP vaccine antigen content are not available. Therefore, product-specific reference preparations may be used.

A.5.1.5 Sterility tests for bacteria and fungi
Each purified monovalent antigen bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2, of the WHO *General requirements for the sterility of biological substances* (39), or by a method approved by the NRA. 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 integrity of L1 protein is a critical quality parameter and should be carefully monitored. The percentage of intact L1 protein of each purified monovalent antigen bulk should be assessed by suitable methods. The purity assay (section A.5.1.2) may also serve to assess the integrity of the L1 monomer. The percentage of intact L1 monomer is the ratio of the intact
L1 monomer to the total protein expressed as a percentage – i.e. intact L1 monomer/(total L1 + total non-L1) x 100.

A.5.1.7 VLP size and structure
The size and structure of the VLPs are to be established and monitored. This test may be omitted for routine lot release once consistency of production has been established, with the agreement of the NRA.

Suitable methods for assessing VLP size and structure include dynamic light scattering, Size Exclusion Chromatography - High Performance Liquid Chromatography (SEC-HPLC) and transmission electron microscopy (TEM).

A.5.1.8 Tests for reagents used during purification or other phases of manufacture
A test should be done to detect the presence of any potentially hazardous reagents used during manufacture, by a method(s) approved by the NRA. This test may be omitted for routine lot release upon demonstration that the process consistently eliminates the reagent from the purified monovalent antigen bulks.

A.5.1.9 Test for residual DNA derived from the expression system
The amount of residual host cell DNA derived from the expression system should be determined in each purified monovalent antigen bulk by suitably sensitive methods. The level of host cell DNA should not exceed the maximum level agreed with the NRA, taking into consideration issues such as those discussed in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (7).

These tests may be omitted for routine lot release upon demonstration that the process consistently inactivates the biological activity of the residual DNA or reduces the amount and size of the contaminating residual DNA from the purified monovalent antigen bulks, subject to the agreement of the NRA.

A.5.1.10 Bovine serum albumin content
If bovine serum is used in mammalian or insect cell cultures for production, residual bovine serum albumin content should be measured and a specification should be set, and approved by the NRA.

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 in terms of its capacity to remove and/or to inactivate adventitious viruses, as described in the Q5A guidelines (43) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). This testing is performed during vaccine manufacturing development or as part of process validation and is not intended as an assessment for lot release.

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

A.6 Control of adsorbed monovalent antigen bulk

A.6.1 Addition of adjuvant
The purified monovalent antigens may be adsorbed onto an adjuvant such as an aluminium salt, in which case the adjuvant and the concentration used should be approved by the NRA. If an alternative or additional adjuvant such as MPL is used, this should also be approved by the NRA.

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

A.6.2 Storage
Until the adsorbed monovalent antigen 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 NRA.

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 NRA.
A.6.3.1 Sterility tests for bacteria and fungi
Each adsorbed monovalent antigen bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2, of the WHO General requirements for the sterility of biological substances (39), or by an alternative method approved by the NRA.

A.6.3.2 Bacterial endotoxins
Each adsorbed monovalent antigen bulk should be tested for bacterial endotoxins by a method approved by the NRA.

If it is inappropriate to test the adsorbed monovalent antigen bulk, the test should be performed on the purified antigen bulk prior to adsorption, subject to the approval of the NRA.

A.6.3.3 Identity
Each adsorbed monovalent antigen bulk should be identified as the appropriate HPV antigen type by a method suitable for distinguishing between HPV types (e.g. an immunological assay). The test for antigen content may also serve as the identity test.

A.6.3.4 Adjuvant concentration
Adsorbed monovalent antigen bulk may be assayed for the content of the adjuvant until production consistency is demonstrated.

A.6.3.5 Degree of adsorption
The degree of adsorption (completeness of adsorption) of each adsorbed monovalent antigen bulk should be assessed, if applicable. This test may be omitted upon demonstration of process consistency, subject to the agreement of the NRA.

A.6.3.6 pH
The pH value of the adsorbed monovalent antigen bulk may be monitored until production consistency is demonstrated, subject to the agreement of the NRA.

A.6.3.7 Antigen content
The antigen content of the adsorbed monovalent antigen bulk should be measured using appropriate methods. If this test is conducted on purified monovalent antigen bulk, it may be omitted from the adsorbed monovalent antigen bulk.

International standards and reference reagents for the control of HPV VLP vaccine antigen content are not available. Therefore, product-specific reference preparations may be used.

**A.7 Control of final 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 that the dose is consistent with that shown to be safe and effective in human clinical trials. Should an adjuvant be added to the vaccine formulation, this adjuvant and the concentration used should be approved by the NRA.

The operations necessary for preparing the final bulk 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 NRA not to impair the safety and efficacy of the vaccine at the concentration used. The final bulk should be stored under conditions shown by the manufacturer to allow it to retain the desired biological activity until it is filled into containers.

**A.7.1 Tests on the final bulk**

All tests and specifications for final bulk, unless otherwise specified, should be approved by the NRA.

**A.7.1.1 Sterility tests for bacteria and fungi**

Each final bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2, of the WHO *General requirements for the sterility of biological substances* (39), or by a method approved by the NRA.

**A.7.1.2 Adjuvant content**

Each final bulk should be assayed for the content of adjuvants.
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 an adjuvant such as MPL is gas chromatography.

Tests for adjuvant on the final bulk may be omitted if conducted on each final lot derived from the final bulk.

**A.7.1.3 Degree of adsorption**

The degree of adsorption to the adjuvant (completeness of adsorption) of each antigen present in each final bulk should be assessed, if applicable (e.g. if the adjuvant is aluminium salts).

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

**A.7.1.5 Potency**

The potency of each final bulk should be assessed with an appropriate in vivo or in vitro method. If an in vivo potency test is used to test final lots, this test may be omitted on the final bulk. The methods for detection of antibodies to HPV VLPs and the analysis of data should be approved by the NRA. The vaccine potency should be compared with that of a reference preparation; the NRA should determine the limits of potency and approve the reference preparation used.

For ethical reasons, it is desirable to apply the 3R principles (reduction, replacement, refinement) to the use of animals, where scientifically appropriate (44).

**A.7.1.6 Osmolality**

The osmolality of the final bulk may be tested. The osmolality test may be omitted if performed on the final lot.
Alternative tests (e.g. freezing point) may be used as a surrogate measure for ionic strength/osmolality.

**A.8 Filling and containers**

The requirements concerning filling and containers given in the WHO *Good manufacturing practices for biological products* (36) 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 NRA with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

**A.9 Control tests on the final lot**

The following tests should be performed on each final lot (i.e. in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers from each final lot by means of validated methods approved by the NRA. All the tests and specifications, including methods used and permitted concentrations, unless otherwise specified, should be approved by the NRA.

**A.9.1 Inspection of containers**

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded and recorded for each relevant abnormality. A limit should be established for the percentage of containers rejected.

**A.9.2 Appearance**

The appearance of the vaccine should be described with respect to its form and colour.

**A.9.3 Identity**

All antigens present in the final lot should be identified 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, as specified in the requirements in Part A, section 5.2, of WHO’s *General requirements for the sterility of biological substances* (39), or by acceptable methods.

### A.9.5 pH and osmolality

The pH value and osmolality of the final lot should be tested. The osmolality test may be omitted if performed on the final bulk. The osmolality test may also be omitted for routine lot release upon demonstration of product consistency, subject to the approval of the NRA.

Alternative tests (e.g. freezing point) may be used as a surrogate measure for ionic strength/osmolality.

### 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 (e.g. LAL) should be performed. However, where there is interference in the test – for instance, because of the addition of an immunostimulant such as MPL – a test for pyrogens in rabbits should be performed.

A suitably validated monocyte activation test may also be considered as an alternative to the rabbit pyrogen test.

The test is conducted until consistency of production is demonstrated, subject to the agreement of the NRA.

### A.9.8 Adjuvant content

Each final lot should be assayed for the adjuvant content, if applicable. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per dose.

### 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 to the adjuvant (completeness of adsorption) of each antigen present in each final vaccine lot should be assessed, if applicable, and the limit should be approved by the NRA.

This test may be omitted for routine lot release upon demonstration of product consistency, subject to the approval of the NRA.

**A.9.11 Potency**
An appropriate quantitative test for potency by an *in vivo* or *in vitro* method should be performed on samples that are representative of each final vaccine lot. The method and the analysis of data from potency tests should be approved by the NRA. The vaccine potency should be compared with that of a reference preparation, and the limits of potency should be agreed with the NRA. The NRA should approve the reference preparation used. If an *in vivo* potency test is used, this test may be omitted on the final bulk. The method of testing for antigen potency in an in vitro test could be quantitative with respect to the antigen content or relative to a reference preparation.

Because of the diversity in the reactivity of vaccines containing HPV VLPs produced by different manufacturing techniques and differences in the adjuvants used for the vaccine formulation, it is unlikely that International Standards will be suitable for the standardization of assays of vaccines from all manufacturers. Consequently, International Standards will not be developed for the potency of each HPV type. Manufacturers should establish a product-specific reference preparation that is traceable to a lot of vaccine, or bulks used in the production of such a lot, which has been shown to be efficacious in clinical trials. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and the reference vaccine should be replaced when necessary. An acceptable procedure for replacing reference vaccines should be in place (45, 46).

**A.9.12 General safety (innocuity) test**
The need to test the final lots of the HPV vaccine for unexpected toxicity (also known as abnormal toxicity) should be agreed with the NRA.

Some countries no longer require this test (47).

A.10 Records
The requirements given in section 8 of the WHO *Good manufacturing practices for biological products* (36) should apply.

A.11 Retained samples
The requirements given in section 9 of the WHO *Good manufacturing practices for biological products* (36) should apply.

A.12 Labelling
The requirements given in section 7 of the WHO *Good manufacturing practices for biological products* (36) 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, bacterial cells, mammalian cells or recombinant baculovirus/insect cells;
- the type of HPV, from which L1 VLP was derived, that is present in the preparation;
- the 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 the WHO *Good manufacturing practices for biological products* (36) should apply. Further guidance is provided in the WHO Model
guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (48).

A.14 Stability testing, storage and expiry date
A.14.1 Stability testing
Adequate stability studies form an essential part of vaccine development. The current guidance on evaluation of vaccine stability is provided in WHO’s Guidelines on stability evaluation of vaccines (49). Stability testing should be performed at different stages of production, namely on single antigen harvests or single harvest pools, purified monovalent antigen bulk, adsorbed monovalent antigen bulk, final bulk, whenever materials are stored before further processing, and final lot. Stability-indicating parameters appropriate to the stage of production should be defined or selected. A shelf-life should be assigned to all in-process materials during vaccine production – particularly intermediates such as single antigen harvests, purified monovalent antigen bulk and final bulk.

The stability and expiry date of the vaccine in its final container, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA using final containers from at least three final lots made from different adsorbed monovalent antigen bulks.

Given the complexity of these multivalent vaccines, other approaches may be used with the approval of the NRA.

Accelerated stability tests may be undertaken to give additional information on the overall characteristics of a vaccine and may also be useful to aid in assessing comparability when the manufacturer plans to change manufacturing.

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 NRAs. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (49). Data should be provided to the NRA according to local regulatory requirements.
The final stability-testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection and sharing of stability data, and criteria for rejecting vaccines(s).

A.14.2 Storage conditions
The final lot should be kept at +2 °C to +8 °C. If other storage conditions are used, they should be fully validated and approved by the NRA. 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.

If a vaccine has been shown to be stable at temperature ranges higher than the approved +2 °C to +8 °C range, it may be stored in a controlled temperature chain (CTC) for a defined period of time, subject to approval by the NRA.

A.14.3 Expiry date
The expiry date should be based on the shelf-life supported by stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of final bulk, date of filling or the date of the first valid potency test on the final lot.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals are inoculated.

Part B. Nonclinical evaluation of recombinant HPV VLP vaccines
Details of the design, conduct, analysis and evaluation of nonclinical studies are available in WHO’s Guidelines on nonclinical evaluation of vaccines (5). Further guidance on the general principles for nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines can be found in a separate WHO document (8). In particular, this guideline should be consulted to determine the need for safety pharmacology and biodistribution studies as well as the extent of characterizing the adjuvant alone, should a novel adjuvant be introduced into the HPV vaccine formulation.
This section will apply to new L1 VLP vaccines containing other HPV types in addition to types 16/18 (± 6/11) contained in the related licensed vaccine, and to new L1 VLP vaccines containing types 16/18 (± 6/11) with and without additional types.

Prior to the clinical testing of any new HPV vaccine in humans, there should be extensive product characterization, proof-of-concept immunogenicity studies, and safety testing in animals. The extent of nonclinical evaluation will depend on the complexity of the vaccine formulation, on a case-by-case basis. The following specific issues should be considered in the context of the development of an HPV L1 VLP-based vaccine.

**B.1 Product characterization and process development**

It is critical that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing and the collection of nonclinical data that may suggest potency and safety in humans. The extent of product characterization may vary according to the stage of development. The vaccine lots used in nonclinical studies should be adequately representative of the formulation intended for use in clinical investigation and, ideally, should be the same lots as those used in clinical trials. If this is not feasible, the lots used in nonclinical studies should be comparable to clinical lots with respect to physico-chemical characteristics and data, stability and formulation.

**B.2 Pharmacodynamic 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 VLP-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 (20, 21). Furthermore, transfer of serum from animals vaccinated with L1 VLP provided protection in nonvaccinated animals challenged with the virus (20–22). The mechanism of protection studies in a murine cervicovaginal model of infection using a surrogate virus or pseudovirion suggests that exudation of systemic neutralizing antibodies into the wound bed and binding to the virus at the basement membrane probably account for the protection (22).

On the basis of these data:
• No further challenge 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 substantially involved as a direct effector mechanism of protection; thus, this aspect does not need further elucidation to support regulatory approval.

It is recommended that the pharmacodynamic properties of an L1 VLP-based vaccine should be assessed through immunogenicity studies (e.g. in rodents, rabbits and/or possibly, in nonhuman primates) which should take into account:

• the evaluation and characterization of the serum neutralizing antibodies induced against each of the HPV L1 VLP types included in the proposed vaccine. If the HPV vaccine contains new types in addition to those in a licensed vaccine or a new vaccine formulation intended for an alternative route of administration (e.g. aerosol), the inclusion of additional arm(s) in the study design may generate supportive data;

• in the case of the inclusion of a specific adjuvant in the vaccine, the added benefit with respect to enhancing desired immune response (humoral and/or cellular, e.g. involvement of T-helper cells or induction of specific memory cells) to be studied in one or more relevant species (8);

• the generation of supportive data with respect to the relative ratio of the antigen and the adjuvant included in the vaccine formulation;

• the evaluation of serum cross-neutralizing antibodies to a broad range of HPV types, not included but related to, those in the proposed vaccine is optional (see C.6)

B.3 Toxicology studies
Toxicology studies should be undertaken with the final vaccine formulation in accordance with the WHO guidance (5). Such studies should be performed in relevant animal models, and should reflect the intended clinical use of the vaccine and may include the administration of doses prior to and during pregnancy (5). Because the target population for the HPV vaccines includes women of childbearing age, reproductive and developmental toxicity studies are required. Some NRAs will require submission of the pivotal data at the time of the marketing authorization application (8). Repeated-dose toxicity studies and developmental toxicity studies should include evaluation of the immune response in a group of vaccinated
animals, in addition to appropriate toxicological endpoints, taking into account existing WHO guidelines (5, 8). Other toxicity studies, such as an inhalation toxicity study, may be required if the vaccine is developed for administration through the aerosol route.

If a novel cell substrate (i.e. a substrate that has not previously been used to produce a licensed human vaccine or biological product) is used for the production of an HPV VLP vaccine, safety aspects – such as potential immune responses elicited by residual host cell proteins – should be investigated in a suitable animal model. Such studies should be undertaken particularly if the final product contains an adjuvant that might enhance responses to low levels of residual proteins.

**Part C. Clinical evaluation of recombinant HPV VLP vaccines**

**C.1 Introduction**

Clinical studies should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (50) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6).

This section refers throughout to L1 VLP vaccines that contain at least HPV types 16 and 18 and considers the clinical data that may be generated both prior to initial licensure and post-licensure in different settings.

Three types of clinical data can provide an indication of the ability of a candidate HPV vaccine to prevent anogenital carcinomas:

- immunological data, with use of immunobridging to support efficacy, as discussed in section C.2;
- virological data, with use of viral persistence data to support efficacy, as discussed in section C.3;
- histological data, with detection of precancerous changes in anogenital epithelia to support efficacy, as discussed in section C.4.

Section C.5 summarizes the type(s) of data that may be used to demonstrate vaccine efficacy in different settings.
These four sections take into account the fact that histological primary efficacy endpoints have diminishing feasibility as a result of the impact of HPV vaccines on the occurrence of infections due to types 16 and 18 and the lower prevalence of other oncogenic HPV types in high-grade intraepithelial neoplasms and cancers. Experience gained with the first two HPV vaccines has supported the use of type-specific viral persistence as an alternative primary efficacy endpoint where there has been no routine HPV vaccination or uptake has been low. Neither histological nor viral persistence endpoints are likely to be feasible for types 16 and 18 in countries that have included HPV vaccines in their routine programmes with high uptake rates. These endpoints are also not expected to be feasible for documenting efficacy against other HPV types that are much less commonly, and sometimes rarely, associated with pre-malignant lesions. Consequently, in some settings an assessment of vaccine efficacy can be based only on immunological data.

In addition:

- Section C.6 considers the data that could support claims for protection against HPV types that are not included in a candidate vaccine (i.e. cross-protection).
- Section C.7 considers the assessment of safety within clinical studies.
- The assessment of antibody persistence, vaccine effectiveness and safety in routine use is considered in section C.8.

### C.2 Immunological data

Naturally acquired neutralizing (i.e. functional) antibody to HPV has been demonstrated to afford some type-specific protection (51). HPV L1 VLP vaccines are thought to protect against persistent viral infection by eliciting neutralizing antibody that can be measured in serum. Studies with intramuscular HPV L1 VLP vaccines have found an overall correlation between antibody levels in serum and in cervical mucosal fluids (52-56). However, the antibody concentrations in serum or at the site of infection that are required for protection are unknown – i.e. no immunological correlate of protection (ICP) has been established for HPV vaccines.

The initial assessment of immune responses to HPV L1 VLP vaccines should be based on measurement of neutralizing antibodies in serum using a pseudovirion-based neutralization
assay. Measurement of antibody concentrations at anogenital sites (e.g. in cervical mucosal fluid) is not required.

C.2.1 Assays
Throughout any one clinical development programme, it is preferable that the same assays for determination of anti-HPV antibody are used and that testing is conducted at a designated central laboratory. Ideally, the same approach should apply to post-licensing investigations of antibody persistence. This uniformity becomes essential within any one study and when attempting to compare immune responses between studies. The assay or assays (if a change in assay during clinical development was unavoidable) that were used to generate the immune response data included in the application dossier should be fully validated. The details and results of the validation exercise(s) should be provided.

In vitro neutralizing antibody assays involve measurement of the inhibition of HPV pseudovirus infection of cultured cells and usually employ type-specific pseudovirions carrying a marker plasmid to allow infected cells to be scored easily. These neutralizing assays require expression plasmid constructs for L1 and L2 for each viral type, and assay standardization relies on use of the same source for these constructs. The *Human papillomavirus laboratory manual* (24) includes a method for HPV neutralizing assays that has shown good inter-laboratory performance.

However, neutralization assays are labour-intensive, technically complex and not currently amenable to high throughput. Therefore, following characterization of the neutralizing antibody response to a candidate HPV vaccine, the use of alternative assay methods that are less technically demanding (e.g. type-specific competitive Luminex-based immunoassay [cLIA] or EIA) may be acceptable subject to demonstrating a strong correlation between the results of these assays and neutralizing assays (57).

Competitive immunoassays utilizing neutralizing monoclonal antibodies that bind to conformational epitopes on L1 are type-specific and sensitive, detect all immunoglobulin classes, and do not measure antibodies to denatured L1 protein (57). However, only a subset of the total anti-VLP antibodies is measured since binding to only one neutralizing epitope is
monitored. Therefore, if these assays are used it should be kept in mind that the results may under-represent the total level of protective antibodies in sera.

VLP-based EIA require the use of conformationally intact VLPs as antigens to ensure type-specificity. These assays detect all antibodies of a specific immunoglobulin class (generally IgG) that bind to the VLPs. While both neutralizing and non-neutralizing antibodies are detected, the correlation with neutralizing assays has been generally good because the strongest host response to vaccines developed to date is to neutralizing epitopes. Laboratories performing HPV serology testing have to prepare and conduct quality control for their own VLPs because no commercial assays are available. The inability of laboratories to have access to the common source key reagents for serology assays imposes significant challenges to standardizing HPV serology results.

International Standards for serum antibodies to HPV 16 and HPV 18 are available to assist in improving comparability of results. Use of the parallel-line method with standards calibrated to the International Standard is described in the Human papillomavirus laboratory manual and has been shown to improve inter-laboratory comparisons (24). Antibody levels should be reported in International Units (IU) for HPV types for which an International Standard is available. It should be kept in mind that the comparison of titres between HPV types is not appropriate. For each assay the lower limit of detection (LLOD) and lower limit of quantitation (LLOQ) should be clearly established, along with a justification of the cut-off applied to differentiate samples that are reported to be seropositive and seronegative.

C.2.2 Characterization of the immune response
During the initial phase of the clinical development programme, it is recommended that the following should be documented for each HPV type in a candidate vaccine:

- immune responses to the vaccine, with a focus on seroconversion rates in subjects who were seronegative prior to vaccination;
- pre- to post-vaccination changes in geometric mean titres (GMTs) and reverse cumulative distributions (these should be presented separately for subjects who were seropositive or seronegative prior to vaccination).
The following should be evaluated in clinical studies, unless the sponsor demonstrates that the candidate vaccine is very similar to a licensed vaccine, so that the same sera collection times and dose regimens may apply:

- The kinetics of the immune response (i.e. changes in antibody levels in response to sequential doses) should be determined to support the timing of the post-vaccination serological primary endpoint.
- There should be an adequate exploration of immune responses to support the number of doses and dose interval selected for various age groups.

Inclusion of an adjuvant, whether novel or already included in licensed vaccine(s), should be supported by data demonstrating enhancement of the immune response to one or more HPV types and by an assessment of the potential clinical significance of the antibody levels achieved in the target age range (8). Since there is no established ICP for HPV vaccines, the potential clinical significance of the effect of an adjuvant could be assessed by comparing antibody levels achieved by the candidate vaccine with and without the adjuvant with those elicited by a licensed vaccine for which efficacy has been documented (see section C.2.3.).

If the adjuvant in a candidate vaccine is not identical to one in any type of licensed vaccine for which there is already substantial information available on the mechanism of immune enhancement, its effect on the humoral and cellular immune response (e.g. involvement of T-helper cells or induction of specific memory cells) should be extensively characterized.

Currently, there is no evidence indicating the need for booster doses after completion of a primary series with HPV L1 VLP vaccines. Nevertheless, this remains an open question until such time as very long-term data on vaccine effectiveness are available, (e.g. more than 10 years), including in subjects who were vaccinated several years prior to sexual debut. Consequently, consideration could be given to a pre-emptive assessment of immune responses to booster doses administered to relatively small subsets at planned intervals following completion of the primary series (58). These data may be provided after initial licensure and may also give an indirect indication of priming by the primary series (see section C.8.1).
It is important for antibody levels to be measured at several pre-planned time points after the final vaccine dose in order to construct antibody decay curves that are sufficient to document the achievement of a plateau. These data can be collected post-licensing during long-term follow-up of antibody persistence in selected vaccinated cohorts (see section C.8.1).

The potential for immune interference between HPV VLP vaccines and other vaccines likely to be given concomitantly should be investigated pre- and/or post-licensing. The WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6) should be consulted regarding the design and analysis of these studies.

C.2.3 Analyses of immune responses in comparative studies

In different studies or within the same study, and depending on circumstances that limit the feasibility of some options, as discussed in the sections that follow, the immune response to a candidate vaccine may be compared with one or more of:

- another HPV vaccine, in which case there may be some HPV types in both vaccines (shared types) and some that are in one vaccine only (unshared types);
- the same candidate vaccine but administered at a different dose, different schedule or in a different population;
- another formulation of the candidate vaccine (e.g. with and without an adjuvant, with variable numbers of HPV types);
- a group that does not receive HPV vaccine (i.e. a group that receives placebo or a non-HPV vaccine).

In each setting, it is recommended that:

- Due to their clinical importance, comparisons of immune responses should strongly support a conclusion that vaccine efficacy against types 16 and 18 is very likely to be comparable to that observed for the initial two HPV vaccines that were developed. For this reason, it is advisable that sample size calculations are based on the power needed to show superiority or non-inferiority (see below) for immune responses to each of these two HPV types.
- The primary analysis population for immune responses to each HPV type is confined to those who are seronegative for the particular HPV type at baseline. Therefore, the
sample size calculations should also take into account the anticipated HPV type-
specific baseline seropositivity in the population under study.

- Primary comparisons should be based on antibody titres in sera obtained at 1 month 
after the final dose of the intended regimen(s) unless antibody kinetic data suggest 
otherwise. If a test regimen consists of a different number of doses from the control 
regimen (e.g. 2 versus 3 doses) or if the last dose is given at a different time point 
(e.g. at 4 months versus 6 months after the first dose), the primary comparison should 
still be based on sera obtained at 1 month (or other time point based on kinetics) after 
the final dose, whenever that occurs. Secondary analyses should compare antibody 
titres measured at predefined time points from the first dose, including a comparison 
once antibody levels have reached a plateau.

C.2.3.1 Comparison with a group that does not receive HPV vaccine

Given the widespread licensing of HPV vaccines and their incorporation into routine 
vaccination programmes in many countries, studies of sexually active men or women that 
include a group that does not receive HPV vaccine will be unacceptable in many settings. 
Comparisons with a group that does not receive HPV vaccine may still be possible in short-
term immunogenicity studies in which subjects are considered to be at low or no risk of HPV 
infection (e.g. prior to sexual debut) and in which all subjects eventually receive active 
vaccination within a protocol-specified timeframe. Nevertheless, before selecting this design, 
the potential need for and value of comparing a candidate vaccine with a control group that 
does not receive HPV vaccine should be given careful consideration and should be balanced 
with the ethical considerations regarding withholding a safe and effective treatment.

Post-vaccination seropositivity rates and seroconversion rates in previously seronegative 
subjects have been very high with the initial HPV vaccines, and these are sensitive endpoints 
for studies that compare vaccinated groups with groups that do not receive HPV vaccine. 
Consequently, these studies should aim to demonstrate superiority of seroconversion rates to 
each HPV type in the candidate vaccine group over the control group that does not receive 
HPV vaccine. The predefined criteria for concluding superiority should take into account the 
type-specific seroconversion rates that have been observed for the initial HPV vaccines for 
which efficacy was demonstrated on the basis of histological and virological data (59, 60).
The same approach is applicable whenever comparing immune responses to HPV types that are included in a candidate HPV vaccine but are not in the control HPV vaccine (i.e. unshared types) since the control group is not vaccinated with respect to these types.

**C.2.3.2 Comparisons between vaccinated groups**

Due to the expectation that post-vaccination seropositivity rates and seroconversion rates will be very high in previously seronegative subjects, these are not sensitive endpoints for discriminating between immune responses in vaccinated groups. Therefore GMT ratios for immune responses to each HPV type that is included in the candidate vaccine(s) and control HPV vaccine(s) (i.e. shared types) are usually used for the primary comparisons between vaccinated groups. HPV type-specific seroconversion rates should be included among the secondary endpoints.

As a general rule, for the purposes of establishing non-inferiority between vaccine groups based on GMT ratios for antibody to individual HPV types, it is suggested that the lower bound of the 95% confidence interval around the GMT ratio (test vs. reference vaccine) should not fall below 0.67. Under certain circumstances, NRAs may consider allowing a lower bound of 0.5. In future, especially if an ICP can be identified or if a sponsor is able to offer a sound rationale, it may be appropriate to reconsider these acceptance criteria. In addition, any marked separations between the reverse cumulative distributions should be discussed in terms of the potential clinical implications, even if these occur only at the lower or upper ends of the curves.

**C.2.4 Immunobridging of vaccine efficacy**

Although there is no ICP for HPV vaccines, it is sometimes appropriate or necessary to use immunobridging to evaluate efficacy against anogenital carcinomas, preneoplastic lesions and genital warts.

Immunobridging involves a demonstration of comparable immune responses between a candidate vaccine and a licensed vaccine that has been selected in accordance with the recommendations made in section C.2.4.1. In this way:

- The HPV type-specific efficacy observed with one HPV vaccine may be bridged to an alternative schedule (e.g. reducing the number of doses, delaying the final dose),
population (e.g. children prior to sexual debut in whom efficacy cannot be assessed) or an extended version (i.e. added HPV types) of that same vaccine.

- The efficacy observed with a specific licensed HPV vaccine may be bridged to a candidate vaccine for all HPV types that are shared between the two vaccines.

The comparison of immune responses should be made in accordance with the recommendations outlined in section C.2.3.2. A successful demonstration of non-inferiority in each age and gender subgroup relevant to the indications approved for the control vaccine could support the same indications for the candidate vaccine – i.e. including cervical, anal and vulvo-vaginal lesions. Nevertheless, when the inference of vaccine efficacy is based only on immunobridging data, individual NRAs may decide to restrict the indications of candidate vaccines compared with those of licensed comparator vaccines.

There may be situations in which the predefined non-inferiority margin is not met for one or more HPV types. Since the implications of such a finding for clinical efficacy cannot be determined, the possible effect on vaccine efficacy and effectiveness will have to be considered on a case-by-case basis, taking into account any possible merits of the candidate vaccine and/or test regimen (e.g. a reduced or more convenient schedule, use in a specific age group that facilitates incorporation into existing routine schedules) as well as the relative importance of the HPV type(s) in question and/or their related cross-reactive types prevalent in an individual NRA’s jurisdiction.

If non-inferiority is not demonstrated for one or more HPV types based on immune responses determined shortly after the last dose, a pre-defined analysis that demonstrates non-inferiority at later time points might be considered as an alternative – e.g. a comparison at 18–30 months after the last vaccine dose, when current experience suggests onset of a plateau effect on the antibody decay curves. However, as time progresses since the last vaccine dose, the difference between GMTs is likely to diminish, reducing the analytical sensitivity. In addition, basing the comparison on antibody levels at 18–30 months after the last dose would considerably delay the initial licensure of a vaccine.

**C.2.4.1 Selection of the comparator vaccine**
It is critically important that immunobridging approaches to assess potential vaccine efficacy are based on appropriate comparator vaccines. In principle, the comparator vaccine should have been licensed on the basis of histological or, at least, virological primary endpoints. However, in some regulatory jurisdictions, it may not be possible to license a candidate vaccine on the basis of immunobridging to the efficacy demonstrated by another HPV vaccine within a specific number of years after the comparator was first licensed. Therefore the selection of comparator vaccine(s) should be discussed with the relevant NRAs of the countries where the candidate vaccine is intended for use. In most instances it will be appropriate to compare the candidate vaccine with a licensed vaccine that contains the same HPV types or as many shared HPV types as possible.

In future, it may be that immune responses to a candidate vaccine cannot be directly compared with those to a vaccine that was previously shown to have efficacy against disease-related endpoints because it is no longer marketed. For example, the original version of a vaccine that was licensed on the basis of histological and/or virological data may have been replaced by a modified version (e.g. with additional HPV types). Using the modified version in immunobridging studies becomes a problem if it does not elicit very similar immune responses to the HPV types in the original version. As a result, there is a risk that modified versions could be less immunogenic and potentially less protective than the original vaccines that were developed. This inescapable fact increases the importance of post-licensure documentation of vaccine effectiveness (see section C.8.2.).

C.2.4.2 Immunobridging in specific circumstances

For the first two HPV vaccines to be developed, immunobridging was used to support their use in children aged 9–15 years. Some NRAs have subsequently accepted immunobridging to support a reduction from 3 to 2 doses in specific age subsets (61-70). In each of these cases, licensure was based on demonstrating non-inferiority of immune responses in children to those in female vaccinees in the age range within which efficacy had been demonstrated following a 3-dose schedule.

In these bridging studies, the immune responses to HPV types 16 and 18 were higher in boys than in girls aged 9–15 years (64, 71). In contrast, immune responses to some HPV types have been lower in men than in women of comparable ages and lower in women aged >
26–45 years compared to women aged 15–26 years (72, 73). Therefore, whenever an immunobridging approach is used, it is relevant to consider possible issues of interpretation based on age range and gender (see further in section C.5).

An immunobridging approach to support use in immunosuppressed populations is complicated by the likelihood of observing lower immune responses compared to those in healthy subjects (74-76). The implications of lower immune responses for vaccine efficacy are uncertain, as an ICP has not been established. NRAs will have to consider whether the anticipation of some degree of benefit in immunosuppressed populations, even if potentially lower than in immunocompetent subjects, is sufficient to support a favourable benefit–risk conclusion.

C.3 Virological data
The reliable determination of HPV infection and viral persistence at anogenital sites requires the use of carefully controlled and standardized methods of sample collection, processing and virus detection.

C.3.1 Sampling
Because HPV is cell-associated, samples must contain cellular material and separate samples must be obtained from each specific anatomical site of interest.

Methods of sample collection that have been validated in large-scale epidemiological studies are recommended. The specific method used (e.g. in terms of number of turns and depth of insertion of the device) should be standardized and adhered to for each study. Ideally, no changes to the method should be made during each study or during the entire clinical development programme. If changes are unavoidable, there should be adequate cross-validation to support the pooling of results obtained with different methods. The collection medium will influence the volume of sample to be extracted as well as the method of extraction. Water or collection medium blanks should be processed and tested along with samples to ensure that no cross-contamination occurs during processing (24).

The standard approach for monitoring HPV in the cervix is for samples to be collected from the ecto- and endo-cervix by clinicians after visualization of the cervix using speculum
examination (24). Although a range of collection devices may be used, they should target the cervical transformation zone and each device should be compatible with the selected collection medium. Alternative methods, such as sampling only from either the ecto-cervix or the endo-cervix and self-sampling by study participants, may be considered if appropriate validation is provided.

C.3.2 Virus detection

The assays used for the determination of virological endpoints in studies intended to support licensure should provide type-specific results with high sensitivity and specificity and should include an internal control to monitor the adequacy of samples (77, 78). The internal control, directed to a cellular DNA sequence, establishes a lower limit for acceptable cell number but will not evaluate the quality of the cellular sample. For instance, it will not distinguish between inflammatory cells (unintended sample) and epithelial cells (intended sample).

Participating in proficiency tests, when available, and obtaining satisfactory results is one step towards ensuring the quality of the assay (78). It should be noted that proficiency tests that use purified DNA will not monitor extraction efficiency, impact of possible PCR inhibitors in samples or competition between types with large differences in copy number. The Human papillomavirus laboratory manual (24) provides guidance on quality assurance and quality control aspects of HPV DNA testing to laboratories performing testing for virological endpoints, in order to help ensure stability and standardization of results over time.

C.3.3 Viral persistence

As discussed under General considerations, the vaccine efficacy studies that were conducted with the first HPV vaccines in sexually active young women demonstrated a close correlation between the primary histological endpoint of high-grade cervical disease and 6-month persistent infection of the cervix with HPV (3, 79-83). On this basis, and taking into account the similarity of disease pathogenesis and progression at cervical and anal sites, viral persistence based on at least two consecutive samples obtained over at least 6 months is an acceptable alternative to histological endpoints at these anatomical sites (3). Nevertheless, HPV type-specific viral persistence is a feasible primary endpoint only when rates of persistent infections in vaccinees known before enrolment or found to be HPV DNA negative
after enrolment for the types in question are sufficiently high to allow studies of reasonable size. This is not expected to be a feasible endpoint for studies in populations in which HPV vaccines have been introduced into routine immunization programmes and in which there has been a very high uptake. It is also not likely to be a feasible endpoint for HPV types that are rarely encountered, regardless of any vaccine usage.

The demonstration of viral persistence should be based on consecutive type-specific HPV DNA positive samples obtained from the same anatomical site over at least 6 months from the time of the first positive result. Because the timing of incident infections after completion of the vaccination series cannot be predicted, an event-driven analysis is often employed. Thus, the primary analysis is conducted when a protocol-defined number of total cases of viral persistence (which may be based on HPV types 16 and 18 or otherwise defined by types) has been accumulated. This total number should be estimated to provide sufficient power to detect meaningful differences between treatment groups. In the primary analysis, the cases of viral persistence should be counted from a predefined period (e.g. at least 2 weeks) after the final dose of the vaccination series. A secondary analysis could be based on counting all cases from the time of the first dose. Protocols should address how to handle sequential results when at least one of a series of samples is negative for HPV DNA but is followed by one or more positive samples.

**C.3.3.1 Comparison with a group that does not receive HPV vaccine**

For the same reasons as discussed in section C.2.3.1, studies of viral persistence that include a group that does not receive HPV vaccine will be unacceptable in many settings. If such a design is still considered acceptable and if incidence rates are sufficiently high to make the study feasible (i.e. due to lack of widespread implementation of HPV vaccination in the regions where the study will be conducted), then a superiority design could be used.

Depending on what is known about incidence rates, there could be co-primary endpoints of viral persistence for each of types 16 and 18 or a single composite primary endpoint based on viral persistence for both types. For candidate vaccines containing additional HPV types, a primary analysis could be based on pooled data for types 16 and 18 with a co-primary or secondary analysis based on viral persistence data pooled for all other HPV types and
supportive analyses of viral persistence for each HPV type. Separate or combined studies addressing cervical and anal sites and/or by gender could be considered.

C.3.3.2 Comparisons between HPV vaccines
As discussed above, it is much more likely that another HPV vaccine containing types 16 and 18, with or without additional types, would have to be administered to the control group. Assuming that the candidate vaccine has good efficacy, the experience obtained with the first two HPV vaccines indicates that few, if any, cases of viral persistence of any HPV type will occur in any reasonable time frame, and it may be necessary to resort to an immunobridging approach for assessment of vaccine efficacy (see C.2.3.2). Nevertheless, whenever viral persistence is assessed among the study endpoints, the following approaches are suggested:

- Although persistent infection due to types 16 or 18 is not likely to be a feasible primary endpoint, all cases should be monitored in case the candidate vaccine has unexpectedly low efficacy (e.g. there could be a negative impact of additional HPV types on the protection against types 16 and 18 afforded by a highly multivalent candidate vaccine).
- For any additional HPV types shared between the candidate and comparator vaccines, supportive analyses should compare viral persistence data pooled across all shared types as well as for each individual type.
- For any HPV types in the candidate vaccine only, viral persistence may be pooled across the additional types, but supportive analyses should be conducted for individual types.

C.4 Histological data
As discussed in sections C.2 and C.3:

- Viral persistence endpoints can be used instead of histological endpoints to support the efficacy of a candidate vaccine.
- The acceptability of a control group that does not receive HPV vaccine is rapidly diminishing.
- The feasibility of using histological endpoints is influenced by the same issues that impact on viral persistence endpoints – i.e. decreasing rates of events due to widespread vaccine use and very low rates of lesions associated with some HPV types regardless of any vaccine usage.
If histological data are to be collected in a study, it is recommended that the endpoints used in the pivotal efficacy studies that were conducted with the two initial HPV vaccines in women and the one in men should be used – i.e. incident high-grade anogenital lesions associated with positivity for oncogenic HPV types (59, 60, 84).

C.5 Evaluation of vaccine efficacy in different settings

This section summarizes approaches to assessing potential vaccine efficacy against anogenital carcinomas in different settings. The following issues should be noted:

- Depending on the local circumstances (i.e. whether any HPV vaccines have been licensed, the types of HPV L1 VLP they contain, and whether they have been incorporated into routine vaccination programmes), individual NRAs may have alternative requirements to the recommendations made in this section.
- For reasons discussed in section C.4., histological endpoints are not considered.
- In all settings, only serological data can be obtained from subjects prior to sexual debut.
- This section does not cover the assessment of efficacy against genital warts. For a candidate HPV vaccine containing types 6 and 11, the considerations regarding whether genital warts is a feasible endpoint are the same as those outlined in section C.3.3.1 for viral persistence as an endpoint. In all other settings, efficacy against genital warts will have to be based on demonstrating similar immune responses to these two HPV types between the candidate vaccine and a suitable control vaccine, as outlined in section C.2.4.

C.5.1 Efficacy against HPV types 16 and 18

Efficacy may be evaluated by one of the following approaches:

- viral persistence under the circumstances described in section C.3.3.1;
- immunobridging to a licensed vaccine, as described in section C.2.4.

C.5.2 Efficacy against other HPV types

An evaluation of efficacy against other HPV types associated with anogenital carcinomas is necessary in the following situations:
i) Addition of one or more HPV type(s) associated with anogenital carcinomas to a licensed vaccine that contains at least HPV types 16 and 18 L1 VLPs. In this case, comparisons should be made with the original licensed vaccine and/or another licensed vaccine that contains as many shared types as possible.

ii) Development of a vaccine *de novo* that contains HPV types 16 and 18 plus additional types, in which case a) all the additional types may already be included in a licensed vaccine or b) one or more of the additional types may not be present in any licensed vaccine. In these cases, comparisons could be made with one or more of the following:

- a group that does not receive HPV vaccine, if this option is still appropriate;
- a licensed vaccine that contains types 16 and 18 but does not include the additional types that are in the candidate vaccine;
- a licensed vaccine that contains as many shared types as possible.

**C.5.2.1 Shared HPV types**

Efficacy may be evaluated by one of the following approaches:

- viral persistence, as described in section C.3.3.2;
- demonstration of non-inferiority of immune responses to HPV types shared between the candidate and comparator vaccines, as described in section C.2.3.2.

**C.5.2.2 Unshared HPV types**

- For HPV types in the candidate vaccine but not in the comparator vaccine, there is the possibility of demonstrating superiority for the candidate vaccine on the basis of a composite viral persistence endpoint (either all additional types or all types), as described in section C.3.3.1. In reality, the incidence of viral persistence due to the unshared types may be so low that it precludes this approach because of the sample size that would be needed.

- If the approach described above is not feasible, the assessment of efficacy of the unshared types can be based only on immunological data. There are no sound interpretive criteria that can be recommended to infer vaccine efficacy. Nevertheless, one approach may be to demonstrate that the seroconversion rate to each unshared type is non-inferior to whichever is lowest of the seroconversion rates to types 16 and
18 in the licensed vaccine group. It is not appropriate to base the comparisons on the GMT ratios because these are known to be very variable between types.

C.5.3 Modification(s) of the mode of use
Comparisons of immunological data, as described in sections C.2.3 and C.2.4, may be used to support licensure in the following settings:

- changes in schedule or age range for a licensed HPV vaccine;
- changes in formulation and/or route of administration of a vaccine that includes L1 VLPs manufactured exactly as for the original version of a licensed vaccine;
- de novo development of a candidate vaccine with a different age range or schedule from that of licensed HPV vaccines, or specifically formulated for a route of administration different from that of licensed HPV vaccines

Additional comments regarding specific situations are provided below.

C.5.3.1 Extending the age range downwards
The use of HPV vaccines from the age of 9 years was supported by immunobridging, as described in section C.2.4.2. In the absence of an ICP that might be applied to interpret long-term antibody persistence data, it has been assumed that vaccination from 9 years of age will provide protection in the years following sexual debut on the basis of observations that initial post-vaccination antibody titres fall to a plateau that is maintained after several years.

If a vaccine is proposed for use from < 9 years of age, it is recommended that the potential value of vaccination even earlier in life should be discussed with NRAs. If this strategy is pursued, it is recommended that immune responses in male and female subjects in the new target age range (e.g. 6 to < 9 years) are directly compared with those in men and women aged 15–26 years – i.e. in the age group in which efficacy has been demonstrated on the basis of histological and virological endpoints. A comparison between children aged < 9 years and those aged 9 to < 15 years should not be the basis for licensure since efficacy has not been established in the latter age range.

C.5.3.2 Extending the age range upwards
Studies using histological or viral persistence endpoints have been conducted in women aged 26–45 years but not in men aged > 26 years. Interpretation of the available data by individual NRAs has varied. As a result, different approaches have been taken to the prescribing information in terms of whether to impose an upper age limit or omit any upper age limit for one or both sexes.

It is not expected that efficacy can be demonstrated in individuals > 26 years of age on the basis of prevention of HPV-associated advanced dysplastic lesions or cervical cancer. As the routine use of HPV vaccines increases, the feasibility of demonstrating efficacy based on viral persistence will decrease. An immunobridging approach may not succeed since immune responses may be naturally lower as age increases. As a result, it is not possible to recommend an approach to support licensure of HPV vaccines for subjects older than 26 years. NRAs must decide on a case-by-case basis whether additional data would be needed to include older subjects in the indications for use, taking into account what seems feasible and the public health considerations applicable to the region.

C.6 Cross-protection

The sponsor may choose to assess the ability of a vaccine to elicit cross-reacting neutralizing antibody against non-vaccine HPV types that are closely related to the types included in a vaccine. However, experience indicates that these data cannot be used to establish the ability of a vaccine to confer cross-protection (85).

Thus far, claims for cross-protection against HPV types related to, but not included in, a vaccine have been based on relatively short-term histological and viral persistence data. Since the assessment of specific epitopes that elicit cross-protection is not part of the HPV L1 VLP vaccine specifications, the degree of cross-protection may be very vaccine-specific. Currently, or in the future, it may not be feasible to demonstrate cross-protection based on virological persistence, yet reliance on immunobridging to a licensed vaccine is not straightforward because:

- Available data suggest that cross-neutralizing antibody may not be predictive for cross-protection.
There would have to be a case-by-case evaluation of the validity of extrapolating cross-protection claims between products based on the possibility that small differences between epitopes could have an impact on cross-protection.

Therefore, if viral persistence cannot be used as an endpoint, it does not appear that future cross-protection claims can be supported.

C.7 Safety
The general considerations for the pre-licensure assessment of safety during clinical studies are those outlined in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (6).

In the specific case of HPV vaccines, it is important that the safety database adequately covers the entire target age range and both sexes, as applicable to the authorized indications, but it is not necessary to generate safety data in each subset that could be sufficient to estimate frequencies of uncommon adverse events. The numbers vaccinated within each sex and age subgroup should be supported by a discussion of any anticipated differences that could preclude assumptions of similar safety profiles according to the characteristics of vaccinees (e.g. if the reactogenicity profile seems to be very different between men and women or between adults and younger subjects).

Regardless of protocol recommendations for studies conducted with sexually active women, numerous pregnancies have been documented in vaccinees. Every effort should be made to estimate the stage of gestation in relation to vaccine doses and to document the outcome of the pregnancy. The conduct of specific studies in pregnant women is not recommended at this time (86).

Assessment of safety in the post-licensure period is discussed below in section C.8.3.

C.8 Post-licensing evaluation
C.8.1 Immunological follow-up
The duration of immunological follow-up to document antibody persistence at the time of initial licensure may be very limited. At the time of licensure, plans should be in place to document antibody persistence to each HPV type in the vaccine in the longer term. These
data should be obtained from subsets of subjects who were initially vaccinated across the entire age range of use and in both sexes. The final duration of follow-up should be agreed with NRAs on the basis of knowledge of antibody decay curves and of the magnitude of the initial immune response to vaccination (see C2.2).

As discussed in C.2.2, since it is not yet known whether additional doses of vaccine after completion of the primary series may be needed to assure prolonged protection, an assessment of immune responses to booster doses at planned intervals following completion of the primary series (58) should be conducted.

C.8.2 Vaccine effectiveness and related issues
It is important to obtain data on vaccine effectiveness and the risk of an HPV type-replacement phenomenon in the post-licensing period. Some NRAs consider that collection of these data is a responsibility of the licence holder. Nevertheless, generation of reliable data usually requires activation of large-scale public health initiatives and disease monitoring on a national or regional basis (e.g. the use of cancer registries to capture the effect of HPV vaccines on rates of HPV-related anogenital carcinomas). Thus, post-licensing commitments that individual NRAs may require from licence holders are expected to relate to cooperative efforts (e.g. public–private partnerships, where these are deemed to be acceptable) with appropriate bodies.

In some regions where routine vaccination against HPV is not yet established there may be specific oncogenic HPV types that are detected relatively frequently (with or without types 16/18 or other types) in association with pre-cancerous anogenital lesions and anogenital carcinomas. Nevertheless, for HPV vaccines that contain the specific type(s) in question, pre-licensure estimates of vaccine efficacy based on histological or viral persistence endpoints may not be available. In such regions, especially when claims for efficacy against these types are based on immunological data only, the responsible NRAs may consider it particularly important to obtain vaccine effectiveness data.

If a licensed vaccine has been granted claims for cross-protection based on relatively short-term histological and viral persistence data (see section C.6.), there should be further follow-up to confirm these claims in the post-licensure period.
C.8.3 Safety monitoring

The general considerations for safety surveillance and for the development of a pharmacovigilance plan are the same as for all other types of vaccines (6).

Where HPV vaccines have been introduced into routine vaccination programmes, they are mostly administered in age groups of children expected to be prior to sexual debut. However, initial catch-up programmes that include older subjects have sometimes resulted in inadvertent vaccination during early pregnancy. Safety surveillance programmes should capture the outcomes of these pregnancies (86).

There may be some instances in which NRAs require specific post-licensing safety studies to be conducted by the licence holder in order to address particular concerns. The design and time frame for reporting these studies should be agreed at the time of initial licensure.

Part D. Recommendations for NRAs

D.1 General recommendations

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (87) and Guidelines for independent lot release of vaccines by regulatory authorities (88) should apply. These recommendations specify that no new biological substance should be released until consistency of batch manufacturing and quality has been demonstrated.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines, should be discussed with and approved by the NRA.

For control purposes, the relevant International Standards currently in force should be obtained for the purpose of calibrating national, regional and working standards (46). The NRA may obtain from the manufacturer the product-specific/working reference to be used for lot release.
Consistency of production has been recognized as an essential component in the quality assurance of recombinant HPV VLP. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as a series of consecutive lots of the vaccine.

D.2 Release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO recommendations (88).

A protocol for the manufacturing and control of recombinant human papillomavirus virus-like particle vaccines, based on the model protocol provided in Appendix 1, and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for the release of a vaccine for use.

A lot release certificate signed by the appropriate NRA official should then 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 WHO recommendations. The certificate should provide sufficient information on the vaccine lot. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 2.

Authors and acknowledgements

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Further changes to document WHO/BS/2015.2252 were then made by the WHO Expert Committee on Biological Standardization, resulting in the present document.
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Appendix 1

Model protocol for the manufacturing and control of recombinant human papillomavirus virus-like particle vaccines

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the manufacturer to the NRA. Information and tests may be added or omitted as necessary with the approval of the NRA.

It is 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 recommendations for a particular product should be provided in the protocol submitted.

The section concerning the final lot must be accompanied by a sample of the label and a copy of the leaflet (package insert) 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 NRA or from the NCL in the country in which the vaccine was produced or released, stating that the product meets national requirements as well as the recommendations in Part A of this document.

1. Summary information on the final lot

International name:

Trade name/commercial name:

Product licence (marketing authorization) number

Country:

Name and address of manufacturer:

Name and address of licence holder, if different:
Final lot

Batch number(s):
  Final lot:
  Final bulk:
Type of container:
Total number of filled containers in this final lot:
Number of doses per container:
Antigen concentration/volume of single human dose:

2. Production information

Batch number of each monovalent bulk (purified and/or adsorbed):
Site of manufacture of each monovalent bulk:
Date of manufacture of each monovalent bulk:
Site of manufacture of adjuvant(s):
Date of manufacture of adjuvant(s):
Site of manufacture of final bulk:
Date of manufacture of final bulk:
Site of manufacture of final lot:
Date of manufacture of final lot:

Date on which last determination of potency was started or date of start of period of validity:
Shelf-life approved (months):
Expiry date:
Storage conditions:
Release date:

A genealogy of the lot numbers of all vaccine components used in the formulation of the final lot will be informative.
The following sections are intended for the reporting of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production. Therefore if any test has to be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

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.

Control of source materials (section A.3)

Cell cultures for antigen production (section A.3.1)

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 product licence:

Manufacturer’s working cell bank (WCB) lot number and preparation date:

Population doubling level (PDL) of manufacturer’s WCB

Date of approval of protocols indicating compliance with the requirements of the relevant monographs and with the product licence:

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):

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**Virus seed lots if a recombinant baculovirus expression vector is used (section A.3.2)** (Sufficient detail should be provided for inoculum intermediates, including the passage level from the master seed and the length and conditions of storage, if any.)

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

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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 product licence:

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 product licence:

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**Each seed lot should be tested for the following:**

**Identity**

Method:

Specification:

Date:

Result:

---

**Bacteria and fungi**

Method:

Media:

Volume inoculated:

Date of start of test:

Date of end of test:

Result:

---

**Mycoplasmas, spiroplasma, entomoplasm and mesoplasma**

Method:

Volume inoculated:
Date of start of test: ____________________________
Date of end of test: ____________________________
Result: ______________________________________

Adventitious agents
Method: ______________________________________
Specification: _________________________________
Date: _________________________________________
Result: ______________________________________

Mycobacterium spp. (if applicable)
Method: ______________________________________
Media: _______________________________________
Volume inoculated: ____________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: ______________________________________

Recombinant baculovirus concentration
Method: ______________________________________
Specification: _________________________________
Date: _________________________________________
Result: ______________________________________

Control cell cultures if mammalian or insect cells are used for production and recombinant viral vector cannot be neutralized, thus interfering with testing.

Provide information on control cells corresponding to each single harvest (section A.3.2.1.5).

Ratio or proportion of control to production cell cultures:
Volume of control cells: _______________________
Dates of observation of cultures: _______________
Percentage rejected for non-specific reasons: __________
Result: ______________________________________

Karyotype
Method: ______________________________________
Probe: _______________________________________
Reference cells: _______________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: ______________________________________
**Identity test by DNA finger printing (if applicable)**

| Method: | |
| Probe: | |
| Reference cells: | |
| Restriction enzymes: | |
| Date of start of test: | |
| Date of end of test: | |
| 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 of start of test: | |
| Date of end of test: | |
| Result: | |

**Tests on supernatant fluids or cell lysates for other adventitious agents (if relevant)**

| Date of sampling from production cell cultures: | |

**Type of simian cells**

| Quantity of sample inoculated: | |
| Incubation temperature: | |
| Date of start of test: | |
| Date of end of test: | |
| Percentage of viable culture at the end: | |
| Result: | |

**Type of human cells**

| Quantity of sample inoculated: | |
| Incubation temperature: | |
| Date of start of test: | |
| Date of end of test: | |
| Percentage of viable culture at the end: | |
| Result: | |

**Type(s) of other cells**

<p>| Quantity of sample inoculated: | |
| Incubation temperature: | |
| Date of start of test: | |</p>
<table>
<thead>
<tr>
<th>Date of end of test:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of viable culture at the end:</td>
<td></td>
</tr>
<tr>
<td>Result:</td>
<td></td>
</tr>
</tbody>
</table>

**Bacteria and fungi**

| Method: |  |
| Media: |  |
| Volume inoculated: |  |
| Date of start of test: |  |
| Date of end of test: |  |
| Result: |  |

**Mycoplasmas**

| Method: |  |
| Volume inoculated: |  |
| Date of start of test: |  |
| Date of end of test: |  |
| Result: |  |

**Single antigen harvests (or pools) (section A.4)**

| Batch number(s): |  |
| Date of inoculation: |  |
| Date of harvesting: |  |
| Volume(s) of cell suspension/paste or supernatant, storage temperature, storage time and approved storage period: |  |

**Culture purity or sterility for bacteria and fungi**

| Method: |  |
| Media: |  |
| Volume inoculated: |  |
| Date of start of test: |  |
| Date of end of test: |  |
| Result: |  |

**Identity of HPV type**

| 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:

Volume inoculated:
Date of start of test:
Date of end of test:
Result:

Purified monovalent antigen bulk (section A.5)

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:
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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 of start of test: ____________________________
Date of end of test: ____________________________
Result: ____________________________

Percentage intact L1 monomer
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ____________________________

VLP size and structure
Report on this is needed until production consistency is demonstrated
Method: ____________________________
Tests for reagents used during purification and other phases of manufacture (if relevant)
Method:
Specification:
Date:
Result:

Residual DNA (if applicable)
Method:
Specification:
Date:
Result:

Bovine serum 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.5.1.11
Method:
Specification:
Date:

Adsorbed monovalent antigen bulk (section A.6)
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 of start of test: ____________________________
Date of end of test: ____________________________
Result: __________________________________________

**Bacterial endotoxins**
Method: __________________________________________
Specification: ___________________________________
Date: ___________________________________________
Result: __________________________________________

**Identity**
Method: __________________________________________
Specification: ___________________________________
Date: ___________________________________________
Result: __________________________________________

**Adjuvant concentration (if relevant)**
Method: __________________________________________
Specification: ___________________________________
Date: ___________________________________________
Result: __________________________________________

**Degree of adsorption (if applicable)**
Method: __________________________________________
Specification: ___________________________________
Date: ___________________________________________
Result: __________________________________________

**pH**
Method: __________________________________________
Specification: ___________________________________
Date: ___________________________________________
Result: __________________________________________

**Antigen content (if applicable)**
Method: __________________________________________
Batch number of reference vaccine and assigned potency: ___________________________________
Specification: ___________________________________
Date: ___________________________________________
Result: 

<table>
<thead>
<tr>
<th><strong>Final bulk</strong> <em>(section A.7)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Batch number:</strong></td>
</tr>
<tr>
<td><strong>Date of manufacture:</strong></td>
</tr>
<tr>
<td>Batch numbers and volumes of adsorbed bulk vaccines used for the formulation of the final bulk vaccine:</td>
</tr>
<tr>
<td>Batch number(s) and volume(s) of bulk alum diluent:</td>
</tr>
<tr>
<td>Volume, storage temperature, storage time and approved storage period:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Bacteria and fungi</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method:</strong></td>
</tr>
<tr>
<td><strong>Media:</strong></td>
</tr>
<tr>
<td>Volume inoculated:</td>
</tr>
<tr>
<td>Date of start of test:</td>
</tr>
<tr>
<td>Date of end of test:</td>
</tr>
<tr>
<td><strong>Result:</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Adjuvants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method:</strong></td>
</tr>
<tr>
<td><strong>Specification:</strong></td>
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<tr>
<td><strong>Date:</strong></td>
</tr>
<tr>
<td><strong>Result:</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Degree of adsorption (if applicable)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method:</strong></td>
</tr>
<tr>
<td><strong>Specification:</strong></td>
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<tr>
<td><strong>Result:</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Preservatives (if applicable)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method:</strong></td>
</tr>
<tr>
<td><strong>Specification:</strong></td>
</tr>
<tr>
<td><strong>Date:</strong></td>
</tr>
<tr>
<td><strong>Result:</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Potency</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>If an in vitro assay of each type is used</em></td>
</tr>
</tbody>
</table>
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Method:
Batch number of reference vaccine and assigned potency: ________________________________
Specification: ________________________________________________________________
Date: ____________________________
Result: ______________________________________________________

If an in vivo assay is used
Species, strain, sex and weight specifications: __________________________________________
Dates of vaccination, bleeding: ______________________________________________________
Date of assay of each type: __________________________________________________________
Batch number of reference vaccine and assigned potency: ________________________________
Vaccine doses (dilutions) and number of animals responding at each dose for each type: ______
ED\textsubscript{50} of reference and test vaccine for each type: _________________________________
Potency of test vaccine versus reference vaccine for each type with 95\% confidence limits of mean: _________________________________
Validity criteria for each type: _______________________________________________________

Osmolality (if applicable)
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ______________________________________________________

Freezing point (if applicable)
Method: ____________________________
Specification: ____________________________
Date: ____________________________
Result: ______________________________________________________

Final lot (section A.9)
Batch number: ____________________________
Date of filling: ____________________________
Type of container: ____________________________
Filling volume: ____________________________
Number of containers after inspection: ____________________________

Appearance
Method: ____________________________
Specification: ____________________________
### Identity (each type)
- **Method:**
- **Specification:**
- **Date:**
- **Result:**

### Bacteria and fungi
- **Method:**
- **Media:**
- **Volume inoculated:**
- **Date of start of test:**
- **Date of end of test:**
- **Result:**

### pH
- **Method:**
- **Specification:**
- **Date:**
- **Result:**

### Osmolality
- **Method:**
- **Specification:**
- **Date:**
- **Result:**

### Freezing point (if applicable)
- **Method:**
- **Specification:**
- **Date:**
- **Result:**

### Preservatives (if applicable)
- **Method:**
- **Specification:**
- **Date:**
- **Result:**

### Pyrogenic substances
- **Method:**
<table>
<thead>
<tr>
<th>Specification:</th>
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<tr>
<td>Date:</td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Adjuvant content**

<table>
<thead>
<tr>
<th>Method:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Specification:</td>
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<tr>
<td>Date:</td>
<td></td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Protein content (or calculated value)**

<table>
<thead>
<tr>
<th>Method:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Specification:</td>
<td></td>
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<tr>
<td>Date:</td>
<td></td>
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<tr>
<td>Result:</td>
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</tbody>
</table>

**Degree of adsorption of each type (if applicable)**

<table>
<thead>
<tr>
<th>Method:</th>
<th></th>
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<tbody>
<tr>
<td>Specification:</td>
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<tr>
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<tr>
<td>Result:</td>
<td></td>
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</tbody>
</table>

**Potency:**

**In vitro assay of each type**

<table>
<thead>
<tr>
<th>Method:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Batch number of reference vaccine and assigned potency:</td>
<td></td>
</tr>
<tr>
<td>Specification:</td>
<td></td>
</tr>
<tr>
<td>Date:</td>
<td></td>
</tr>
<tr>
<td>Result:</td>
<td></td>
</tr>
</tbody>
</table>

**If an in vivo assay is used**

<table>
<thead>
<tr>
<th>Species, strain, sex and weight specifications:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dates of vaccination, bleeding:</td>
<td></td>
</tr>
<tr>
<td>Date of assay of each type:</td>
<td></td>
</tr>
<tr>
<td>Batch number of reference vaccine and assigned potency:</td>
<td></td>
</tr>
<tr>
<td>Vaccine doses (dilutions) and number of animals responding at each dose for each type:</td>
<td></td>
</tr>
<tr>
<td>ED$_{50}$ of reference and test vaccine for each type:</td>
<td></td>
</tr>
</tbody>
</table>
Potency of test vaccine versus 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: 

Certification by the manufacturer
Name of Head of Quality Control (typed) _________________________

Certification by the person from the control laboratory of the manufacturing company taking overall responsibility for the production and quality control of the vaccine

I certify that lot no. ___________________ of recombinant human papillomavirus virus-like particle vaccine, whose number appears on the label of the final containers, meets all national requirements and/or satisfies Part A\(^1\) of the WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines (2015).\(^2\)

Signature __________________________________________________

Name (typed) ____________________________________________

Date ____________________________________________________

\(^1\) With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

Certification by the NRA
If the vaccine is to be exported, attach the NRA Lot Release Certificate (as shown in Appendix 2), a label from a final container and an instruction leaflet for users.
Appendix 2

Model NRA Lot Release Certificate for recombinant human papillomavirus virus-like particle vaccines

Certificate No.________________________

The following lot(s) of recombinant human papillomavirus virus-like particle vaccine produced by ____________________________ in ____________, whose numbers appear on the labels of the final containers, meet all national requirements and Part A of the WHO’s Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines (____) and complies with WHO Good manufacturing practices for pharmaceutical products: main principles; Good manufacturing practices for biological products; and Guidelines for independent lot release of vaccines by regulatory authorities.

The release decision is based on _____________________________________________

The certificate may include the following information:

- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container used;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and expiry date;
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate;
- certificate number.

The Director of the NRA (or other appropriate authority)
Name (typed) _______________________________________________________

Signature _________________________________________________________

Date ______________________________________________________________

1 Name of manufacturer.
2 Country of origin.
3 If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.
4 With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.
9 Evaluation of the summary protocol, independent laboratory testing and/or procedures specified in a defined document etc., as appropriate.