Annex 4

Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines

Replacement of Annex 1 of WHO Technical Report Series, No. 962

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Recommendations published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO 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 WHO Recommendations are made only on condition that such modifications ensure that the product 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 intended to provide additional guidance to manufacturers and NRAs.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CIN</td>
<td>cervical intraepithelial neoplasia</td>
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<tr>
<td>CIN2–3</td>
<td>CIN grades 2 or 3</td>
</tr>
<tr>
<td>CIN2+</td>
<td>cervical intraepithelial neoplasia grade 2 or worse</td>
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<tr>
<td>cLIA</td>
<td>competitive Luminex immunoassay</td>
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<tr>
<td>CMI</td>
<td>cell-mediated immunity</td>
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<tr>
<td>DCVMN</td>
<td>Developing Countries Vaccine Manufacturers Network</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
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<tr>
<td>GCP</td>
<td>good clinical practice</td>
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<td>GMC</td>
<td>geometric mean concentration</td>
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<td>GMT</td>
<td>geometric mean titre</td>
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<td>HPV</td>
<td>human papillomavirus</td>
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<tr>
<td>IARC</td>
<td>WHO International Agency for Research on Cancer</td>
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<tr>
<td>ICP</td>
<td>immune correlate of protection</td>
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<tr>
<td>IFPMA</td>
<td>International Federation of Pharmaceutical Manufacturers &amp; Associations</td>
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<tr>
<td>IU</td>
<td>International Unit(s)</td>
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<tr>
<td>LAL</td>
<td>limulus amebocyte lysate</td>
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<tr>
<td>LOD</td>
<td>lower limit of detection</td>
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<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
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<tr>
<td>MCB</td>
<td>master cell bank</td>
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<tr>
<td>MOI</td>
<td>multiplicity of infection</td>
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<tr>
<td>MPL</td>
<td>monophosphoryl lipid A</td>
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<tr>
<td>NAT</td>
<td>nucleic acid amplification technique</td>
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<tr>
<td>NCL</td>
<td>national control laboratory</td>
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<tr>
<td>NRA</td>
<td>national regulatory authority</td>
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<tr>
<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>PDL</td>
<td>population doubling level</td>
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<tr>
<td>RBC</td>
<td>red blood cell</td>
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<tr>
<td>RRP</td>
<td>recurrent respiratory papillomatosis</td>
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<tr>
<td>SAGE</td>
<td>WHO Strategic Advisory Group of Experts</td>
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<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SEC–HPLC</td>
<td>size-exclusion chromatography – high-performance liquid chromatography</td>
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<tr>
<td>spp.</td>
<td>species</td>
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<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
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<tr>
<td>VLP</td>
<td>virus-like particle</td>
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<td>WCB</td>
<td>working cell bank</td>
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Introduction

WHO Guidelines to assure the quality, safety and efficacy of recombinant human papillomavirus (HPV) virus-like particle (VLP) vaccines were first adopted by the WHO Expert Committee on Biological Standardization in 2006 (1) and were based largely on experience gained from clinical trials undertaken on the first two licensed HPV vaccines.

The factors 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 end-points for clinical evaluation of new prophylactic HPV vaccines.

A series of meetings was convened by WHO to review the scientific evidence needed to initiate and inform the revision process. These meetings were attended by experts from around the world involved in the research, manufacture, licensing/authorization, control-testing and release of HPV vaccines. Participants were drawn from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry, and included representatives of the WHO Global HPV LabNet – an initiative that worked towards the 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 end-points for evaluating the clinical efficacy of candidate HPV vaccines. The first meeting held was in February 2013 and considered issues relating to the development and evaluation of clinical end-points for trials of new HPV vaccines and other issues to be addressed in the proposed revision. At a meeting held at the WHO International Agency for Research on Cancer (IARC), Lyon, France in September 2013 a Working Group discussed whether it might be appropriate to consider using a virological end-point – rather than a disease end-point such as cervical intraepithelial neoplasia (CIN) grade 2 or worse (CIN2+) – as the primary end-point for future clinical efficacy trials, and the circumstances under which immunobridging trials might be sufficient for licensure (2, 3). A third meeting held at WHO headquarters in November 2013 reviewed and discussed the outcomes of the IARC scientific meeting on appropriate clinical end-points, reviewed vaccines currently in the development pipeline, and assessed regulatory and laboratory needs for licensing the vaccines (4).

Major issues addressed in these resulting WHO Recommendations include updates of:

- terminology;
- general considerations and other sections to reflect the up-to-date development of HPV vaccines;
- the section on international standards and reference preparations;
- Part A, in order to include bacteria as a cell substrate;
- Part B, in order to include a new subsection highlighting tests 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 end-points in future trials;
- appendices.

Additional changes have also been made to bring the document into line with other WHO Recommendations, Guidelines and guidance documents published since the 2006 adoption of the WHO Guidelines on recombinant HPV VLP vaccines (1).

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 their quality, safety and efficacy.

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 (for example, 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 below may be relevant and may be taken into consideration during vaccine development.

This document should be read in conjunction with other relevant WHO guidance such as that on nonclinical (5) and clinical (6) evaluation of vaccines. Other WHO guidance, such as that on 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. 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. However, in rare instances HPV infection persists. If persistent infection with high-risk types is not detected and treated then progression to invasive carcinoma may occur at the site of infection. The interval between 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 – namely HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Group 1) – and an additional type as probably carcinogenic – namely HPV type 68 (Group 2A) (14). HPV type 66 was formerly classified as oncogenic but recent findings have significantly weakened the evidence for this. Additional data on rare HPV types currently considered possibly oncogenic to

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1 The terms oncogenic/oncogenicity and carcinogenic/carcinogenicity are both used in the scientific literature on HPVs. In this document the terms oncogenic/oncogenicity are used.
humans (Group 2B) suggest that some, but not all, could be upgraded (18). The
distribution and prevalence of the above 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, with HPV type 16
representing an even higher fraction (90%) of HPV-positive cancers of the anus
than is the case for 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 are
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 RRP cases, and for 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 against 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 the former WHO Guidelines to produce these WHO Recommendations has been driven by the experience acquired in using the first two licensed HPV L1 VLP vaccines in many countries, and by the development of an extended version of one of these vaccines to include additional HPV types. These vaccines are made up of recombinant protein L1 VLPs and contain adjuvant to enhance their immunogenicity. 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 final formulation. The three 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) with each dose delivering 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 with each dose delivering 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 (for example, 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 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 by NRAs for the specific production system.

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 – namely groups that received placebo or another vaccine with no potential to protect against HPV. The primary end-points 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 (CIN grades 2 or 3 (CIN2–3) and adenocarcinoma in situ). Secondary end-points 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, the WHO 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 to 13 years – that is, before the age of initiation of sexual activity and exposure to HPV (11).

The initial product licences were for 3-dose schedules (0, 1 or 2 months and 6 months). Subsequently the European Medicines Agency approved: (a) a 2-dose schedule for the bivalent vaccine for females aged 9–14 years; and (b) a 2-dose schedule for the quadrivalent vaccine for females aged 9–13 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 *S. cerevisiae* containing L1 expression plasmids. The licensure of this 9-valent vaccine was based on demonstration of efficacy against the additional HPV types (using a composite histological end-point) and a comparison of immune responses to 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 vaccine 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 as used in these WHO Recommendations. They may have different meanings in other contexts.

**Adjuvant**: a substance or a combination of substances used in conjunction with a vaccine antigen to enhance (for example, 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 (for example, ampoules or vials) should be representative of the pool of cells from which they are taken and should be frozen on the same day following the same procedure and 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 are 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 end-points:** combine two or more single end-points in one outcome to demonstrate overall treatment effects. Subjects who have experienced any of the events specified in the single end-points are considered to have experienced the composite end-point (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 (for example, with baculovirus expression vector) and grown in parallel for the purpose of serving as test material.

**Expression construct:** 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 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 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.

**Final lot:** a collection of sealed final containers of finished vaccine that is homogeneous with respect to the risk of contamination during the filling process. All of the final containers must therefore have been filled from a single vessel of final bulk in one working session.
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 (for example, serum antibodies) and amount above which an individual is most probably protected. For many vaccines, functional antibodies above a threshold geometric mean titre (GMT) or geometric mean concentration (GMC) serve as an ICP.

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 recombinant baculovirus 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 MCB 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 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 prior to purification.

**Working cell bank (WCB):** a quantity of well-characterized cells of animal or other origin, derived from the MCB 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 WCB 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.

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**Part A. Manufacturing recommendations**

**A.1 Definitions and international reference materials**

**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 (for example, types 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 materials**

International Standards for HPV types 16 and 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 the 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, the United Kingdom.

The WHO Catalogue of International Reference Preparations 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 WHO good manufacturing practices for pharmaceutical products: main principles (35) and WHO 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 (for example, deep sequencing) allow for the entire construct to be examined, while others (for example, 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 – for example on 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 their routine use in production or may include samples taken specifically for this purpose.

A.3.1.1 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, section 5.2 of the WHO General requirements for the sterility of biological substances (39), 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. WCBs 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 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). It is important to show that the cell banks are free from bacteria, fungi, mycoplasmas, mycobacterium species (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 (for example, arboviruses).

Insect viruses have not been well characterized compared with other potential adventitious agents, and there is therefore less information about them – and specifically about 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 technique (NAT) tests such as polymerase chain reaction (PCR) and other nonspecific 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 strategy chosen for testing (7). 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 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 the WHO 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, and on the construction, 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 using 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 passage 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 using 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 of the WHO General requirements for the sterility of biological substances, sections 5.2 (39) and 5.3 (40) 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,
the 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 the 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 section A.4.2.1.1 below). 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 (for example, 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 such as guinea-
pigs and/or mice. For details of these 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: (a) degenerate NAT for whole virus families, with analysis of the
amplicons by hybridization, sequencing or mass spectrometry; (b) NAT
with random primers followed by analysis of the amplicons on large
oligonucleotide microarrays of conserved viral sequencing or digital
subtraction of expressed sequences; and (c) 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 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 below 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 Part A of the WHO General requirements for the sterility of biological substances, sections 5.2 (39) and 5.3 (40) – as well as freedom from adventitious viruses.

Detailed guidance on detecting bovine viruses in serum for establishing MCBs and WCBs 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 guidance provided on 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, while 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 mycoplasmal 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 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, the use of human serum albumin 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 (for example, 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 systems 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 at least 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 by the end of the test period and any losses should be due to nonspecific or accidental reasons.

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 cell line as used for the production of virus, but not the same batch of cells as those used for the production of vaccine. In addition, 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 cm²
per ml of pooled fluid. At least one culture vessel of each kind of cell culture should remain un-inoculated 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 by the end of the test period and any losses should be due to nonspecific or accidental reasons.

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 (for example, isoenzyme analyses), cytogenetic tests (for example, for chromosomal markers) and tests for genetic markers (for example, DNA fingerprinting or 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 tests for sterility and adventitious agents, as described below in sections
A.4.4.2.2 and A.4.4.2.4, are not performed immediately then 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 also 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 (for example, 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 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 the 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 section A.4.2.1.1 above).

Additional testing for specific adventitious viruses may be performed, for example by 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 reassembly of VLPs. The entire process (sequence of process steps) used for the purification of the VLPs should be appropriately validated, as described above 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 justified, 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 (for example, 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 for 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 methods 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 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 section A.6.3.7 below) 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 (see section A.5.1.2 above) 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 – that is, 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 carried out to detect the presence of any potentially hazardous reagents used during manufacture, using 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  Tests 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 present in 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 then residual bovine serum albumin content should be measured and a maximum permitted concentration 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 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. This testing
is performed during vaccine manufacturing development or as part of process validation and is not intended as an assessment for lot release.

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

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 justified, 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 using 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 (for example, 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 adjuvant content 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 testing of 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 above. 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 justified, 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 adjuvant content.

Where aluminium compounds are used, the amount of aluminium should not exceed 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 content 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 (for example, if the adjuvant is aluminium salts).

This test may be omitted upon demonstration of 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 (Replacement, Reduction, 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 (for example, freezing point) may be used as surrogate measures for ionic strength/osmolality.

A.8 Filling and containers
The requirements concerning filling and containers given in 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 the vaccine.

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 (that is, 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 tests and specifications, including methods used and permitted concentrations, unless otherwise justified, 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 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.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.

An alternative test (for example, 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 (for example, the limulus amebocyte lysate (LAL) test) should be performed. However, where there is interference in the test – for example, because of the addition of an immunostimulant such as MPL – a test for pyrogens in rabbits should be performed.

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 adjuvant content, if applicable. Where aluminium compounds are used, the amount of aluminium should not exceed 1.25 mg per single human 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 WHO good manufacturing practices for biological products (36) should apply.

A.11  **Retained samples**

The requirements given in WHO good manufacturing practices for biological products (36) should apply.

A.12  **Labelling**

The requirements given in 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 VLPs were derived) 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;
- any special dosing schedules.

A.13  **Distribution and transport**

The requirements given in 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. Current guidance on the evaluation of vaccine stability is provided in the WHO 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 aid in assessing comparability when the manufacturer plans to change aspects of 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 the NRA. 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 in accordance with 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 vaccine(s).

A.14.2 Storage conditions

The final lot should be kept at 2–8 °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 2–8 °C range, it may be stored in a controlled temperature chain 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 the WHO 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 Guidelines document (8). In particular, this latter document should be consulted to determine the need for safety, pharmacology and bio-distribution studies, as well as the extent of characterizing of the adjuvant alone, should a novel adjuvant be introduced into the HPV vaccine formulation.

The guidance given in this current section will also apply to new L1 VLP vaccines containing other HPV types in addition to the 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 crucially important that vaccine production processes are appropriately standardized and controlled to ensure consistency in manufacturing, and in the collection of nonclinical data that may indicate 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 physicochemical characteristics and data, stability and formulation.

B.2 Pharmacodynamic studies

There is no adequate, relevant animal model for HPV infection as 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 cottontail rabbit 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 VLPs provided protection in non-vaccinated animals challenged with the virus (20–22). One mechanism-of-protection study in a murine cervicovaginal model of infection using a surrogate virus or pseudovirion has indicated 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 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 (for example, in rodents, rabbits and/or possibly in non-human 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 (for example, aerosol), the inclusion of an 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 – for example, 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 section C.6 below).

B.3 Toxicology studies
Toxicology studies should be undertaken with the final vaccine formulation in accordance with 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 end-points, 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 (that is, 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 then 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 as discussed in section C.2 below;
- virological data as discussed in section C.3 below;
- histological data as discussed in section C.4 below.

Section C.5 summarizes the type(s) of data that may be used to demonstrate vaccine efficacy in different settings.

These four sections (C.2–C.5) take into account the fact that histological primary efficacy end-points 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 end-point where there has been no routine HPV vaccination or where uptake has been low. Neither histological nor viral-persistence end-points 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 end-points are also not expected to be feasible for documenting efficacy against other HPV types that are much less commonly, and sometimes rarely, associated with premalignant 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 (that is, cross-protection).
- Section C.7 considers the assessment of safety within clinical studies.
- Section C.8 considers the assessment of antibody persistence, vaccine effectiveness and safety in routine use.

C.2 Immunological data

Naturally acquired neutralizing (that is, 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— that is, no immune 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 (for example, 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) 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 WHO 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 (for example, type-specific competitive Luminex immunoassay (cLIA) or EIA) may be acceptable subject to demonstration of 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 EIAs require the use of conformationally intact VLPs as antigens to ensure type specificity. These assays detect all antibodies of a specific immunoglobulin class (generally immunoglobulin G) 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 approaches for their own VLPs because no commercial assays are available. The inability of laboratories to access common key source reagents for serology assays presents significant challenges to the standardizing of HPV serology results.

International Standards for serum antibodies to HPV type 16 and HPV type 18 are available to help improve the comparability of results. The use of the parallel-line method with standards calibrated to the International Standard is described in the WHO Human papillomavirus laboratory manual (24) and has been shown to improve inter-laboratory comparisons. 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 quantification (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 (that is, changes in antibody levels in response to sequential doses) should be determined to support the timing of the post-vaccination serological primary end point.
- 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 below).

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 (for example, 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 (for example, 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 below).

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 below).

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 below – 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 (for example, with and without an adjuvant or with variable numbers of HPV types);

- data obtained from a group that does not receive HPV vaccine (that is, a group that receives either a placebo or a non-HPV vaccine).

In each case, it is recommended that:

- Due to their clinical importance, comparisons of immune responses should strongly support a conclusion that vaccine efficacy against HPV types 16 and 18 is very likely to be comparable to that observed for the two initially developed HPV vaccines.

- 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 (for example, 2 versus 3 doses) or if the last dose is given at a different time point (for example, at 4 months versus 6 months after the first dose) then 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 (for example, prior to sexual debut) and in which all subjects eventually receive active vaccination within a protocol-specified time frame. 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 ethical considerations regarding the withholding of a safe and effective vaccine.

Post-vaccination seropositivity rates and seroconversion rates in previously seronegative subjects have been very high with the initial HPV vaccines, and these are sensitive end-points 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 (that is, 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 end-points 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) (that is, shared types) are usually used for the primary comparisons between vaccinated groups. HPV type-specific seroconversion rates should be included among the secondary end-points.

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 versus 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 below 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 (for example, reducing the number of doses or delaying the final dose) or population (for example, children prior to sexual debut in whom efficacy cannot be assessed), or to an extended version of that same vaccine (that is, with added HPV types);
- 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 above 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 – that is, 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 (for example, a reduced or more convenient schedule, or 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 predefined analysis that demonstrates non-inferiority at later time points might be considered as an alternative – for example, 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 crucially 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 end-points. 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 in which 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 end-points 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 (for example, 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 highlights the vital importance of post-licensure documentation of vaccine effectiveness (see section C.8.2 below).

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 (55, 61–69). 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, 70). 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 (71, 72). Therefore, whenever an immunobridging approach is used, it is relevant to consider possible issues of interpretation based on age range and gender (see section C.5 below).

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 (73–75). 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 risk–benefit 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 (for example, 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-cervix 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, or 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 end-points 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 (76, 77). 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 (77). 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 WHO Human papillomavirus laboratory manual (24) provides guidance on quality assurance and quality control aspects of HPV DNA testing to laboratories performing testing for virological end-points to help ensure stability and standardization of results over time.

C.3.3 Viral persistence

As discussed above 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 end-point of high-grade cervical disease and 6-month persistent infection of the cervix with HPV (3, 78–82). 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 end-points at these anatomical sites (3). Nevertheless, HPV type-specific viral persistence is a feasible primary end-point only when rates of persistent infections in vaccinees who were HPV DNA negative at study baseline for the types in question are sufficiently high to allow for studies of reasonable size. This is not expected to be a feasible end-point 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 end-point 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 statistical power to detect meaningful differences between treatment groups. In the primary analysis, the cases of viral persistence should be counted from a predefined period (for example, 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 discussed above 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 (that is, 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 end-points of viral persistence for each of HPV types 16 and 18 or a single composite primary end-point based on viral persistence for both types. For candidate vaccines containing additional HPV types, a primary analysis could be based on pooled data for HPV 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, 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. Nevertheless, whenever viral persistence is assessed among the study end-points, the following approaches are suggested:

- Although persistent infection due to HPV types 16 or 18 is not likely to be a feasible primary end-point, all cases should be monitored in case the candidate vaccine has unexpectedly low efficacy (for example, there could be a negative impact of additional HPV types on the protection against HPV 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 above in sections C.2 and C.3:

- Viral-persistence end-points can be used instead of histological end-points 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 end-points is influenced by the same issues that affect viral-persistence end-points – that is, 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 end-points used in the pivotal efficacy studies that were conducted with the two initial HPV vaccines in women and with the one in men should be used – that is, incident high-grade anogenital lesions associated with positivity for oncogenic HPV types (59, 60, 83).

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 (that is, 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 above in section C.4, histological end-points 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 end-point are the same as those outlined in section C.3.3.1 for viral persistence as an end-point. 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:

- 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.
- De novo development of a vaccine that contains HPV types 16 and 18 plus additional types, in which case all the additional types may already be included in a licensed vaccine, or one or more of the additional types may not be present in any licensed vaccine. Comparisons could be made with one or more of the following: (a) a group that does not receive HPV vaccine, if this option is still appropriate; (b) a licensed vaccine that contains HPV types 16 and 18 but does not include the additional types that are in the candidate vaccine; (c) 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 above 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 above 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 above 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 HPV 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 above 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 above 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 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 (for example, 6 to < 9 years) are directly compared with those in men and women aged 15–26 years – that is, in the age group in which efficacy has been demonstrated on the basis of histological and virological end-points. 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 end-points 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 to 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 use in 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. Thus far, claims for cross-protection against related HPV types 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. 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 end-point, 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. However, it is not necessary to generate sufficient safety data to estimate frequencies of uncommon adverse events in each subset. The numbers vaccinated within each sex and age subgroup should be supported by discussion of any anticipated differences that could preclude assumptions of similar safety profiles according to the characteristics of vaccinees (for example, 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 among 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. Specific studies in pregnant women are not recommended at this time (85).

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

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, the generation of reliable data usually requires activation of large-scale public health initiatives and disease monitoring on a national or regional basis (for example, the use of cancer registries to capture the effect of HPV vaccines on rates of HPV-related anogenital carcinomas). Thus, the post-licensing commitments that individual NRAs may require from licence holders are expected to relate to cooperative efforts with appropriate bodies (for example, public–private partnerships, where these are deemed to be acceptable).

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 HPV 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 end-points 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 above), 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 vaccine (6).

Where HPV vaccines have been introduced into routine vaccination programmes they are mostly administered to children 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 (85).
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 (86) and Guidelines for independent lot release of vaccines by regulatory authorities (87) 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 or working reference to be used for lot release.

Consistency of production has been recognized as an essential component in the quality assurance of recombinant human papillomavirus virus-like particle vaccines. In particular, the NRA should carefully monitor production records and quality control test results for clinical lots, as well as for a series of consecutive lots of the vaccine.

D.2 Official 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 (87).

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 and/or 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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The third draft was prepared by Dr M. Lennon (Ferguson), Consultant, Horning, the United Kingdom; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr R. Sheets, Consultant, Silver Spring (MD), the USA; Dr T.Q. Zhou, World Health Organization, Switzerland; and other members of the WHO drafting group, taking into consideration the discussions of an informal consultation on WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines held in Geneva, Switzerland, 11–12 November 2014 and attended by: Dr J. Chen (Developing Countries Vaccine Manufacturers Network (DCVMN) representative), INNOVAX, China; Dr G. Coleman, Health Canada,
Canada; Dr C. D.R.Z. Blades, Agência Nacional de Vigilância Sanitária, Brazil; Mr. C.H. Damasceno Cabral (DCVMN representative), Instituto Butantan, Brazil; Dr V.D. Tsu (Representative of other organizations), PATH, the USA; Dr J. Dillner, Karolinska Institute, Stockholm, Sweden; Dr R. Forsythe (International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) representative), GlaxoSmithKline Biologicals, Belgium; Dr R. Herrero, International Agency for Research on Cancer, Lyon, France; Dr C. Jiang (DCVMN representative), Biotechnology Company for High and New Technology, China; Dr W. Kong (DCVMN representative), Biotechnology Company for High and New Technology, China; Dr J. Kukimoto, National Institute of Infectious Diseases, Japan; Dr M. Lennon (Ferguson), Consultant, Horning, the United Kingdom; Dr X. Liang (DCVMN representative), Shanghai Zerun Biotechnology Co. Ltd., Shanghai, China; Dr L. Markowitz, Centers for Disease Control and Prevention, the USA; Dr D. Nardelli-Haefliger, University Hospital, Lausanne, Switzerland; Dr P. Neels, Consultant, University of Namur, Belgium; Dr E. Nkansah (African Vaccine Regulatory Forum representative), Food and Drugs Authority, Ghana; Dr S.Y. Oh, National Institute of Food and Drug Safety Evaluation, Republic of Korea; Dr S. Phumiamorn, Ministry of Public Health, Thailand; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr A. Saah (IFPMA representative), Merck Research Laboratories, the USA; Dr L. Santos Ramalho Evangelista, Agência Nacional de Vigilância Sanitária, Brazil; Dr J. Schiller, National Cancer Institute, the USA; Mr U. Shaligram (DCVMN representative), Serum Institute of India Ltd, India; Dr R. Sheets, Consultant, Silver Spring (MD), the USA; Dr L. Shi (DCVMN representative), Shanghai Zerun Biotechnology Co., Ltd, China; Dr R. Simalango, Directorate of Drug and Biological Product Evaluation, Indonesia; Dr F. Struyf (IFPMA representative), GlaxoSmithKline Biologicals, Belgium; Dr Y. Sun, Paul-Ehrlich-Institut, Germany; Dr I. Uhnnoo, Public Health Agency of Sweden, Sweden; Dr E. Unger, Centers for Disease Control and Prevention, the USA; Dr J.L. Valdez Reyes, Comisión para la Protección contra Riesgos Sanitarios, Mexico; Dr Y. Wang, National Institutes for Food and Drug Control, China; Dr D. Wilkinson, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu (DCVMN representative), INNOVAX, Xiamen, China; and Dr U. Fruth, Dr I. Knezevic, Dr O.C. Lapujade and Dr T.Q. Zhou, World Health Organization, Switzerland.

The fourth draft was prepared by Dr M. Lennon (Ferguson), Consultant, Horning, the United Kingdom; Dr M. Powell, Medicines and Healthcare Products Regulatory Agency, the United Kingdom; Dr T.Q. Zhou, World Health Organization, Switzerland and other members of the WHO drafting group following comments received from Dr J. Dillner, Karolinska Institute, Sweden; Dr R. Herrero, International Agency for Research on Cancer, France;
Dr I. Kukimoto, National Institute of Infectious Diseases, Japan; Professor D. Nardelli-Haefliger, University Hospital, Lausanne, Switzerland; Dr A. Saah, Merck Research Laboratories, the USA; Dr J. Schiller, National Cancer Institute, the USA; Drs F. Struyf and R. Forsythe, GlaxoSmithKline Biologicals, Belgium; Dr I. Uhnoo, Public Health Agency of Sweden, Sweden; Dr E. Unger, Centers for Disease Control and Prevention, the USA; Dr D. Wilkinson, National Institute for Biological Standards and Control, the United Kingdom.

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Further changes were subsequently made to document WHO/BS/2015.2252 by the WHO Expert Committee on Biological Standardization.
References


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 here. 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.

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: ____________________

**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.

**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 such as
albumin or serum): ____________________________

Virus seed lots if a recombinant baculovirus
expression vector is used (section A.3.2)
Sufficient detail should be provided on 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: ____________________________
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: ________________________________

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, entomoplasma 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 nonspecific reasons: __________________________
Result: __________________________

Karyotype

Method: __________________________
Probe: __________________________
Reference cells: __________________________
Date of start of test: __________________________
Date of end of test: __________________________
Result: __________________________

Identity test by DNA fingerprinting (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
Quantity of sample inoculated: ______________________________
Incubation temperature: ___________________________________
Date of start of test: _____________________________________
Date of end of test: _____________________________________
Percentage of viable culture at the end: ________________________
Result: ________________________________________________

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 (for example, 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: ______________________________

Date: ______________________________

Result: ______________________________

**Protein purity (add PAGE photographs)**

Method: ______________________________

Specification: ______________________________

Date: ______________________________

Result: ______________________________
### Protein content

<table>
<thead>
<tr>
<th>Method</th>
<th>Specification</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
</table>

### Antigen content (if applicable)

<table>
<thead>
<tr>
<th>Method</th>
<th>Specification</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
</table>

### Ratio of antigen:protein content (if applicable)

<table>
<thead>
<tr>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
</table>

### Bacteria and fungi

<table>
<thead>
<tr>
<th>Method</th>
<th>Media</th>
<th>Volume inoculated</th>
<th>Date of start of test</th>
<th>Date of end of test</th>
<th>Result</th>
</tr>
</thead>
</table>

### Percentage intact L1 monomer

<table>
<thead>
<tr>
<th>Method</th>
<th>Specification</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
</table>

### VLP size and structure

*Report on this is needed until production consistency is demonstrated*

<table>
<thead>
<tr>
<th>Method</th>
<th>Specification</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
</table>

### Tests for reagents used during purification and other phases of manufacture (if relevant)

<table>
<thead>
<tr>
<th>Method</th>
<th>Specification</th>
</tr>
</thead>
</table>
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: __________________________________________

Date: __________________________________________
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: ________________________________

Final bulk (section A.7)
Batch number: ________________________________
Date of manufacture: ________________________________
Batch numbers and volumes of adsorbed bulk vaccines used for the formulation of the final bulk vaccine: ________________________

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

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

---

**Bacteria and fungi**

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

---

**Adjuvants**

Method: ________________________
Specification: ________________________
Date: ________________________
Result: ________________________

---

**Degree of adsorption (if applicable)**

Method: ________________________
Specification: ________________________
Date: ________________________
Result: ________________________

---

**Preservatives (if applicable)**

Method: ________________________
Specification: ________________________
Date: ________________________
Result: ________________________

---

**Potency**

*If an in vitro assay of each type is used*

Method: ________________________
Batch number of reference vaccine and assigned potency: ________________________
Specification: ________________________
Date: ________________________
Result: ________________________
If an in vivo assay is used
Species, strain, sex and weight specifications: ____________________________
Dates of vaccination, bleeding: ____________________________
Date of assay of each type: ____________________________
Batch number of reference vaccine and assigned potency: ____________________________
Vaccine doses (dilutions) and number of animals responding at each dose for each type: ____________________________
$ED_{50}$ 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: ____________________________
Date: __________________________
Result: __________________________

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 (if applicable)
Method: __________________________
Specification: __________________________
Date: __________________________
Result: __________________________

Freezing point (if applicable)
Method: __________________________
Specification: __________________________
Date: __________________________
Result: __________________________

Preservatives (if applicable)
Method: __________________________
Specification: __________________________
Date: __________________________
Result: __________________________
Pyrogenic substances
  Method: ________________________________
  Specification: ________________________________
  Date: ________________________________
  Result: ________________________________

Adjuvant content
  Method: ________________________________
  Specification: ________________________________
  Date: ________________________________
  Result: ________________________________

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

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

Potency
  If an in vitro assay of each type is used
    Method: ________________________________
    Batch number of reference vaccine and assigned potency: ________________________________
    Specification: ________________________________
    Date: ________________________________
    Result: ________________________________
  
  If an in vivo assay is used
    Species, strain, sex and weight specifications: ________________________________
    Dates of vaccination, bleeding: ________________________________
    Date of assay of each type: ________________________________
    Batch number of reference vaccine and assigned potency: ________________________________
Vaccine doses (dilutions) and number of animals responding at each dose for each type: ______________________________
ED$_{50}$ of reference and test vaccine for each type: ______________________________
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 omission 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 2015 WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines.$^2$

Signature ______________________________
Name (typed) ______________________________
Date ______________________________

**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.

---

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

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 ______________________1 in ______________________2, whose numbers appear on the labels of the final containers, meet all national requirements3 and Part A4 of the 2015 WHO Recommendations to assure the quality, safety and efficacy of recombinant human papillomavirus virus-like particle vaccines5 and comply with WHO good manufacturing practices for pharmaceutical products: main principles;6 WHO good manufacturing practices for biological products;7 and Guidelines for independent lot release of vaccines by regulatory authorities.8

The release decision is based on ______________________9

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);

---

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.
- 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 ________________________________________________________________