Chapter 1 - Introduction

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1.1 PURPOSE AND TARGET AUDIENCE OF THE MANUAL

This interactive manual was developed by the Global Network of National Human Papillomavirus Reference Laboratories (HPV LabNet) based on knowledge and experience gained through international collaborative studies over the past several years, to update the first edition of the HPV laboratory manual.

The World Health Organization (WHO) formed the HPV LabNet in 2005 to assist in global standardization of HPV testing methods required for cervical cancer elimination efforts, including establishing the laboratory support required for implementation and monitoring of human papillomavirus (HPV) vaccination programmes, HPV testing, HPV serology and HPV research. The following manual aims to fulfill these requirements by discussing the role of laboratories in supporting HPV surveillance and vaccination impact monitoring and providing:

- a brief summary of the biology and natural history of HPV and the worldwide burden of HPV-associated diseases.
- the taxonomy and risk association of identified HPV types and putative novel types.
- guidance on laboratory quality assurance (QA).
- guidance on specimen collection and handling for HPV testing.
- guidance on nucleic acid extraction
- an overview of the actual HPV detection and typing methods.
- a summary of HPV serology methods and applications.
- a description of international standards (IS) and secondary standards for HPV testing and their appropriate use.
- guidance on data management.
- considerations when establishing an HPV laboratory.

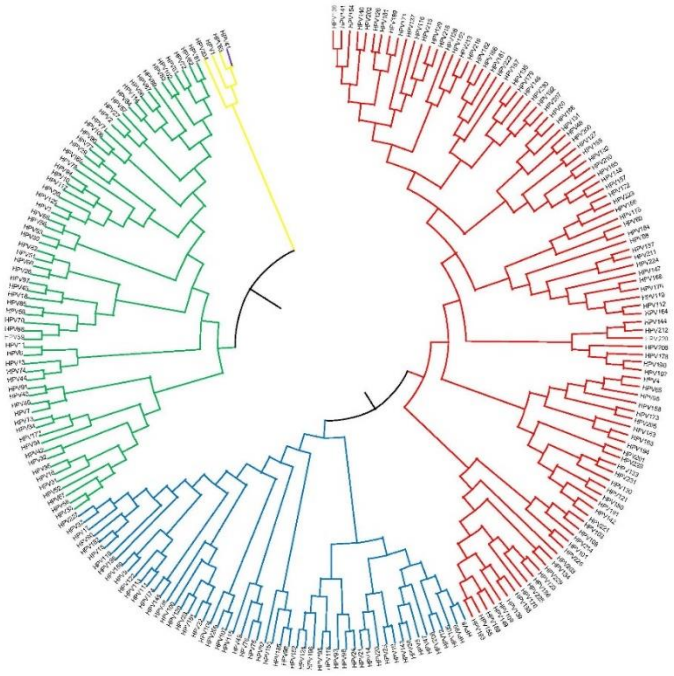
The manual should be useful for all audiences involved in development and implementation of HPV vaccines, particularly those involved in generating or using HPV laboratory data, as well as for laboratories performing cervical screening (HPV testing) and other HPV researchers. This manual is a living document that will be amended in the light of future advances made in the area, and future global experience of HPV screening, HPV laboratory surveillance and vaccination monitoring.

1.2 BIOLOGY AND NATURAL HISTORY OF HPV

HPVs are a group of more than 400 closely related non-enveloped double-stranded deoxyribonucleic acid (DNA) viruses in the Papillomaviridae family.[1] Out of these, more than two hundred are officially established by the International HPV Reference Center (See Chapter 2 for more information about HPV classification).

Individual HPVs are referred to as “types”, distinguished based on their genomic sequence, and numbered in order of discovery. The L1 gene is the region that is most conserved between individual HPV types and is used to form phylogenetic trees used in taxonomy (**Figure 1-1**). The term “genus” is used for the higher order clusters that are named using the Greek alphabet. Within genus, the smaller clusters are referred to as “species” and are named by number.[2]

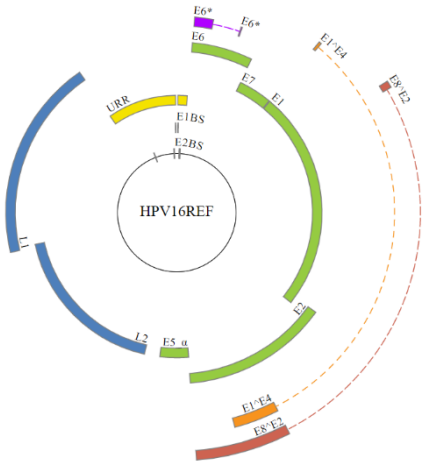
Figure 1-1: Phylogenetic tree comprising all official HPV types established until 2024-10-02 (available at https://www.hpvcenter.se/human_reference_clones/).



Alpha, beta, gamma, mu and nu papillomaviruses are presented in green, blue, red, yellow and purple colors, respectively. The phylogenetic tree is based on the L1 part of the genome. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model[3] and analyses were conducted in MEGA7.[4]

HPVs infect epithelial cells of the skin and mucosal surfaces. The circular genome, approximately 8.0 kilobases (kb), is enclosed in a protein shell made from the major (L1) and minor (L2) capsid proteins resulting in virions approximately 55 nm in diameter. All the coding information is contained in one of the two DNA strands. There are seven open reading frames (ORF) encoding several known viral proteins. The six "early" proteins are E1, E2, E4, E5, E6 and E7. An example of the coding regions for proteins of HPV type 16 can be found at **Figure 1-2**.

Figure 1-2: HPV 16 open reading frames (complete genome is 7906 bp).

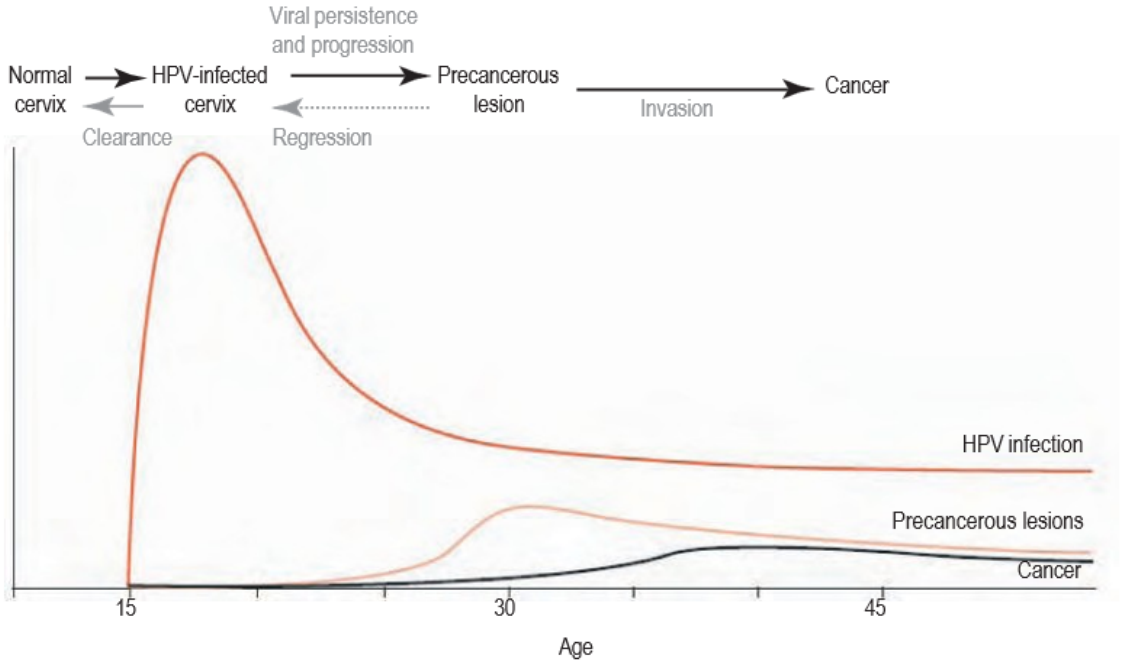


Transcripts encoding the early proteins are detected in the basal and suprabasal epithelial cells in the early portion of the viral replication cycle and encode proteins that interact with the host cell machinery to allow viral replication and transcription to occur. As a result of their function in the normal viral life cycle, the E6 and E7 proteins are also the major viral oncogenes, playing a central role in cell immortalization and transformation. The L1 ORF encodes the major capsid protein (55 kDa) that makes up most of the virus protein shell and its exposed surface. The L2 ORF encodes the minor capsid protein (77 kDa) that contributes a smaller percentage of the capsid mass but plays an important role in encapsidating the viral genome. L2 is not significantly exposed to the surface in intact virions. Expression of the "late" structural L1 and L2 genes is restricted to differentiating epithelium where viral assembly occurs. When expressed in cultured cells, the L1 protein, or L1 and L2 proteins, will self-assemble into structures resembling HPV, called virus-like particles (VLPs).

Approximately 35 HPV types are known to infect the human genital mucosa. They can be grouped as "not oncogenic", "probably/possible oncogenic" or "oncogenic" based on their epidemiologic association with cancer (See Chapter 2, Section 2.3: Risk association). The non-oncogenic types such as HPV6 and HPV11 are associated with genital warts or condyloma acuminate and recurrent respiratory papillomatosis. Oncogenic types such as HPV16 and HPV18 are associated with low- and high-grade cervical intraepithelial lesions (HSIL) and invasive cancer.

Transmission of mucosa-tropic types occurs primarily through sexual contact. HPV is the most common viral sexually transmitted infection, with estimates that up to 75% of sexually active people are infected at some time in their life. The peak age for HPV infection is in the first few years following sexual debut. HPV prevalence in the population decreases in older age as most genital HPV infections resolve without symptoms (typically within 12–18 months). Some infections persist, and persistent infection is more likely to be associated with abnormal cytology and cancer precursors. Invasive cancer is a rare outcome, many years (generally decades) after infection.

Figure 1.3 Diagram representing the natural history of HPV and age-specific prevalence of HPV-associated cervical disease in women [Adapted, by permission, from Schiffman & Castle, 2005].[5]



As HPV does not induce cell necrosis and infection is confined to epithelial surfaces, it is relatively protected from the host immune system. Antibodies to type-specific L1 protein conformational epitopes are detectable in less than 70% of HPV-exposed individuals. Clearance of HPV is associated with a cell-mediated response.

The low-titer HPV antibodies resulting from natural infection can serve as a measure of exposure in the population, but are not useful diagnostically, due to the low rate of seroconversion and antibody persistence after HPV clearance. HPV serology plays an increasingly important role in evaluating new HPV vaccines, reduced dosing schedules, and novel methods of vaccine administration.

HPV cannot be cultured by conventional methods and is a cell-associated virus; therefore, HPV infection is monitored indirectly by detection of HPV DNA in a cellular sample obtained from a particular anatomic site. It should be kept in mind that detection of HPV DNA usually indicates current infection, but surface contamination cannot be excluded. Similarly, failure to detect HPV DNA does not exclude HPV infection, as low-level infections, sampling errors and infections at other anatomic sites cannot be excluded.

1.3 THE BURDEN OF THE DISEASE CAUSED BY HPV

HPV has been associated with more than 90% of cervical cancers.[6, 7] While the type distribution in cervical cancer does vary somewhat worldwide, HPV16 and HPV18 are the most prevalent types, being found in more than 70% of samples from cervical cancer around the world.[8]

The causal relationship between HPV and cervical carcinoma has provided the incentive for the development of vaccines that prevent infection with HPV. Preventing oncogenic HPV infection prevents HPV-associated cancers and pre-cancers. Currently four vaccines are prequalified by WHO, all target HPV16 and HPV18.Cervarix (GlaxoSmith Kline), Cecolin (Xiamen Innovax) target HPV 16 and 18. Gardasil (Merck) includes HPV 6 and 11 and Gardasil 9 (Merck) targets an addition 5 oncogenic types, HPV 31, 33, 45, 52 and 58.

Cervical cancer is the second leading cause of cancer mortality among women in developing countries (**Figure 1-4**). The disease burden contributes to worldwide health inequity, as 80% of cervical cancer deaths occur in under-resourced countries (**Figure 1-4**).[9] Cervical cancer control ideally includes primary prevention with HPV vaccination and secondary prevention through organized screening programmes with associated follow up and treatment of the detected pre-cancerous lesions. Uptake of HPV vaccines remains slow due to cost and vaccine supply issues. Cervical cancer screening programmes have not been effectively implemented in some areas of the world where they are most needed, due to barriers of cost, trained personnel, and infrastructure. While HPV testing as primary screening method is recognized to have optimal sensitivity, many countries continue using cytology as the primary screening method, which is known to be less sensitive and more subjective.

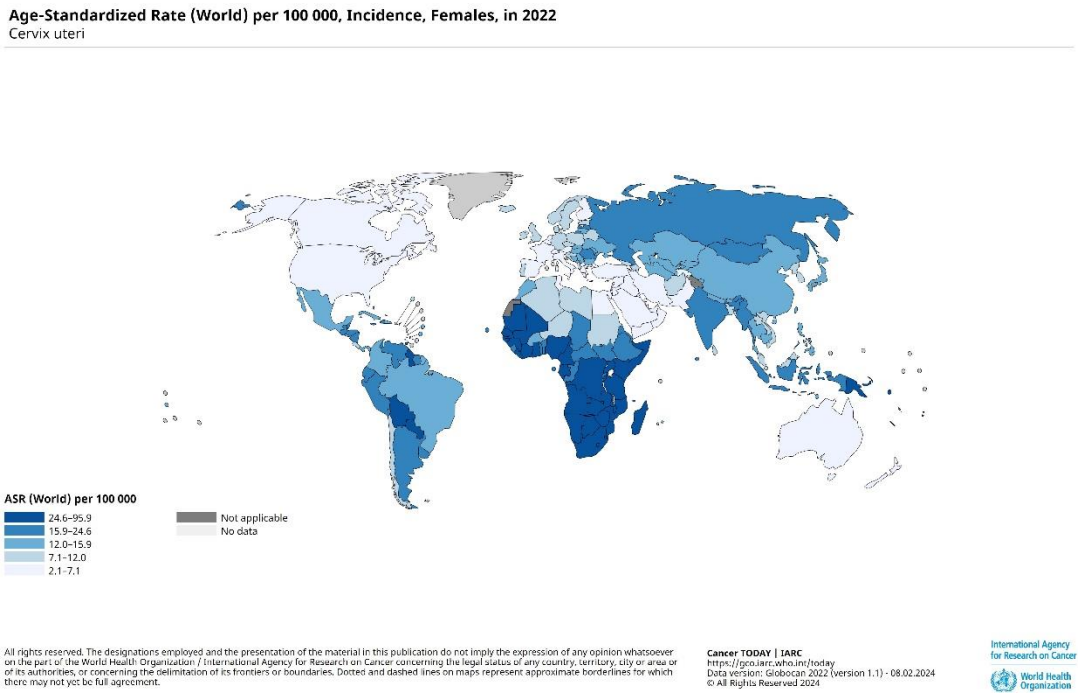
WHO has issued a global call to eliminate cervical cancer as a public health problem, that is to reach and maintain an incidence rate < 4 per 100 000 women. (https://www.who.int/initiatives/cervical-cancer-elimination-initiative, last accessed October 1, 2024) Achieving that goal within the next century requires each country to meet three targets by 2030

- vaccination: 90% of girls fully vaccinated with the HPV vaccine by the age of 15.
- screening: 70% of women screened using a high-performance test by the age of 35, and again by the age of 45.
- treatment: 90% of women with pre-cancer treated and 90% of women with invasive cancer managed.

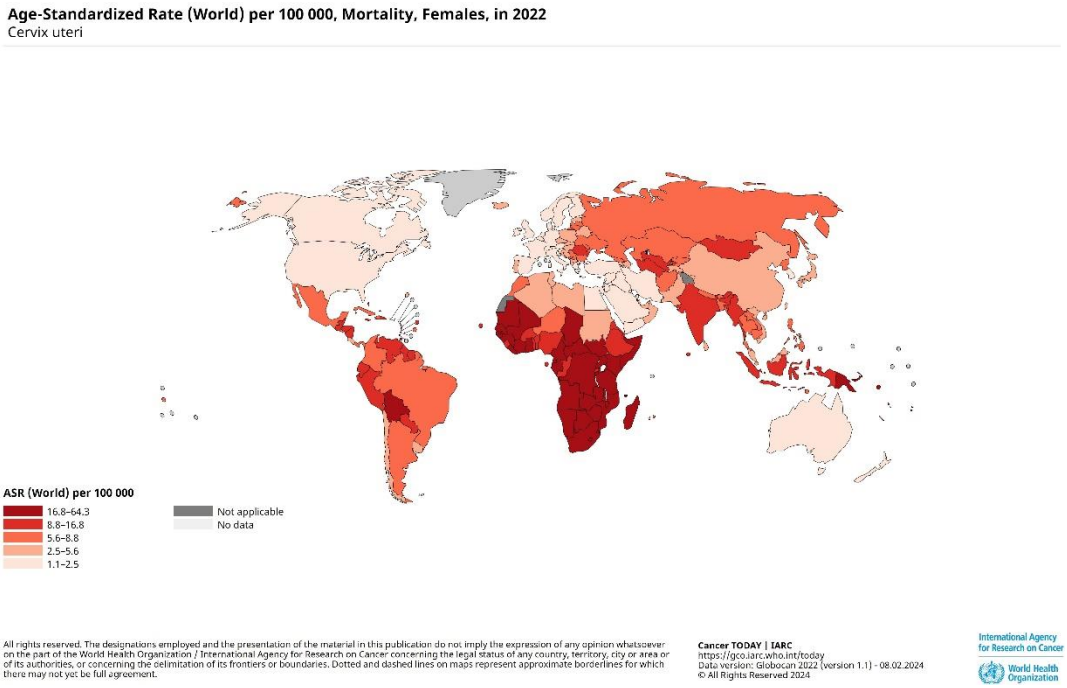
In addition to cervical cancer, HPV is associated with anogenital and oropharyngeal malignancies affecting both males and females. A recent report conducted by the ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre, https://hpvcentre.net/statistics/reports/XWX.pdf, last accessed October 1, 2024) concluded that while essentially all cervical cancer is HPV-associated, other cancers of anal vulva, vaginal, penile, oropharyngeal, oral cavity and laryngeal cancer are HPV-associated to varying degrees (**Table 1-1**).

Figure 1-4 Age-standardised incidence (A) and mortality rates (B) of cervical cancer by country in 2020.

A)



B)



Data are from the GLOBOCAN database, collated by the International Agency for Research on Cancer and hosted by the Global Cancer Observatory (https://gco.iarc.fr/today/en/dataviz/maps-heatmap?mode=population)

Table 1.1 Burden of cervical cancer and other HPV-related cancers

	World	High income countries	Low and middle income countries
Burden of cervical cancer			
Annual number of new cervical cancer cases	662,301	62,809	595,115
Annual number of cervical cancer deaths	348,874	26,800	319,584
Standardized incidence rates per 100,000 population			
Cervical cancer	14.1	7.5	15.9
Anal cancer			
Men	0.51	0.85	0.41
Women	0.57	1.3	0.37
Vulva cancer	0.83	1.6	0.59
Vaginal cancer	0.36	0.34	0.36
Penile cancer	0.79	0.65	0.81
Oropharyngeal cancer			
Men	1.9	3.3	1.5
Women	0.39	0.79	0.29
Oral cavity cancer			
Men	5.8	5.6	5.8
Women	2.3	2.4	2.2
Laryngeal cancer			
Men	3.5	3.5	3.5
Women	0.45	0.58	0.41

Globocan 2022 estimates; World Bank Classification. Adapted and with permission from Bruni L, et al. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. Summary Report 10 March 2023, last accessed October 2, 2024)

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