California Association
for
Medical Laboratory Technology

Distance Learning Program

Papillomaviruses and Cervical Cancer

Course # DL-979

by
Lucy Treagan, Ph.D.
Prof. Biol. Emerita
University of San Francisco

Approved for 2.0 CE
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39656 Mission Blvd.       Phone: 510-792-4441
Fremont, CA  94539       FAX:    510-792-3045

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**COURSE NAME:** PAPILLOMAVIRUSES AND CERVICAL CANCER  
**COURSE #:** DL-979

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2. The objectives of this Distance Learning course were met.  
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   5 4 3 2 1

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Papillomaviruses and Cervical Cancer
Course #: DL-979
Level of Difficulty: Intermediate
2.0 CE

Lucy Treagan, Ph.D.
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OBJECTIVES
Upon completion of this course the participant will be able to:
1. Summarize the principal characteristics of human papillomaviruses (HPVs), including their structure and classification.
2. Explain the link between HPV infection and cervical cancer.
3. Discuss the role of HPVs as etiological agents of skin and genital warts.
4. List various clinical conditions associated with HPV infections.
5. Outline the pathogenesis of HPV infection.
6. Describe host and viral factors that contribute to progression of HPV infection to malignancy.
7. Summarize current diagnostic methods and treatment options.
8. Contrast the principal characteristics of the two vaccines developed for prevention of HPV infection.
9. Discuss the potential effect of HPV vaccination on the incidence of cervical cancer

INTRODUCTION
Papillomaviruses are small DNA viruses that infect mucosal and cutaneous epithelium and cause benign hyperproliferative lesions recognized as warts. These viruses are widely spread in the environment. Infection with papillomaviruses is common in humans and in many animal species, including rabbits, cows, dogs, dolphins, and porpoises. Papillomaviruses are highly specific for their respective hosts. Cutaneous types of HPVs infect the skin of the hands and feet causing formation of warts. Mucosal types infect the lining of the mouth, throat, respiratory tract, and anogenital epithelium. Genital, respiratory, and conjunctival papillomas are among several manifestations of these infections. In most cases, the infection is cleared following activation of the host immune response against the virus. Occasionally, the lesions do not regress and can progress to cancer under appropriate environmental conditions (1). The association with malignancies led to classification of several human papillomavirus types as group 1 carcinogens by the International Agency for Research in Cancer.

HISTORICAL BACKGROUND
The infectious origin of skin warts was long suspected and was eventually proven in the 19th century by experimental transmission as well as by accidental inoculation of healthy subjects (2). Genital warts, however, were considered to be manifestations of common venereal diseases.
Experimental transmission of genital warts to human subjects demonstrated that an infectious agent was involved.

The connection between genital warts and cervical cancer was slow to emerge. In the middle of the 19th century Rigoni Stern, an Italian pathologist, made a suggestion based on epidemiological observations that cervical cancer may have an infectious origin. Various agents, including herpes viruses, were subsequently considered as candidates. In 1977, zur Hausen and his colleagues proposed that some human papillomaviruses were responsible for cervical cancer. They showed that certain HPVs that infect anogenital skin could be isolated from cervical cancers and from cell lines derived from such cancers (3). Subsequent epidemiological studies utilizing DNA hybridization techniques established that most cases of cervical cancer, as well as a number of other anogenital and head and neck cancers, could be attributed to the so-called high-risk human papillomaviruses.

PAPILLOMAVIRUSES: CLASSIFICATION AND PRINCIPAL CHARACTERISTICS

Classification

Papillomaviruses are currently classified in a separate viral family, the *Papillomaviridae*. More than 200 types of human papillomaviruses have been recognized on the basis of DNA sequence data that show genomic differences. Approximately 106 of these genotypes are well characterized. The remaining isolates are partially characterized as potential new genotypes. Recently, a division of *Papillomaviridae* into 16 genera was introduced, based on phylogenetic relationships among human papillomaviruses. Most papillomaviruses belong to either the alpha or the beta genus. The alpha genus includes mucosal/genital papillomaviruses and some cutaneous genotypes. The beta genus comprises genotypes associated mainly with skin infections in immunosuppressed patients. The remaining virus genotypes are grouped into genera gamma, mu, and nu. An alternate classification is based on clinical characteristics of HPVs. According to this classification human papillomaviruses are grouped into 4 categories based on the site of infection (skin or genital) and the probability that the infection will eventually progress to malignancy. HPV genotypes involved in malignancies are known as high-risk types.

**Clinical classification of HPVs (data from reference 3)**

<table>
<thead>
<tr>
<th>Risk of Cancer</th>
<th>Site of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin</td>
</tr>
<tr>
<td>High-Risk (flat lesions)</td>
<td>HPV 5</td>
</tr>
<tr>
<td></td>
<td>HPV 8</td>
</tr>
<tr>
<td>Low-Risk (warty lesions)</td>
<td>HPV 1</td>
</tr>
<tr>
<td></td>
<td>HPV 2</td>
</tr>
</tbody>
</table>

- HPV 8 infection poses cancer risk primarily in immunosuppressed patients or in association with a condition known as epidermodysplasia verruciformis.

Close to 30 genital HPV types are considered high risk. Among these genotypes, infection with HPV 16 accounts for over 50% of cervical cancers. HPV 18 is the next most prevalent papillomavirus genotype isolated from cases of cervical cancer. HPV types 18, 31, and 45 account for an additional 25% to 30% of cervical cancer cases.
Structure of viral particle

Papillomaviruses are small, non-enveloped viruses that contain a double-stranded, circular DNA molecule of approximately 8000 base pairs. The DNA is associated with histones, the basic proteins found in cell nuclei. The viral genome is surrounded by a protein shell (capsid) composed of 72 subunits called capsomers. The capsomers contain at least two proteins, L1 and L2. Of these, L1 is the major structural protein. Five copies of this protein are present in each capsomer. The virion (viral particle) capsid also contains approximately 12 copies of the minor structural protein, L2. The protein subunits are assembled into a highly symmetrical, icosahedral (20 sided) particle. When examined by electron microscopy the virus particle resembles a golf ball.

Viral genome

Like all small viruses the papillomaviruses have a limited coding ability and therefore depend on the host cell to provide factors necessary for viral replication. The number of proteins encoded by the HPV genome is not greater than 10.

The genome is functionally divided into three regions. One segment of the genome has no coding ability. It contains the origin of viral replication and the regulatory elements concerned with transcription. Two other genome regions contain clusters of viral genes, known as the early and the late genes. The early DNA coding region is primarily involved with the replication of the viral genome and with cell transformation. The late genes encode the structural proteins found in the viral capsid.

Viral proteins encoded by late (L) genes

The L1 and L2 structural viral proteins are capable of self-assembly into a symmetrical icosahedral capsid. When viral DNA and both L proteins are present, the DNA is packaged within the viral capsid. The major L1 protein will self-assemble into a non-infectious pseudo-viral particle, which is highly immunogenic. The packaging of the viral genome, however, requires the presence of both capsid proteins. These characteristics of HPV structural proteins have proven to be of major importance in vaccine development.

Viral proteins encoded by early (E) genes

The early proteins are numbered from E1 through E7. These proteins are not incorporated into the viral capsid. They play a critical role in the replication and transcription of the viral genome. In addition, a major function of these proteins is to maintain a replication-competent environment in infected cells since the replication cycle of HPVs is critically dependent on the host cell DNA synthesis machinery. Proteins coded by HPV early genes facilitate viral replication in differentiated squamous epithelial cells, which are normally growth-arrested.

A considerable amount of information is available regarding the functions of some of the early proteins (described in the next section).

E1 and E2 proteins

Viral DNA replication is primarily mediated by E1 and E2 proteins together with cellular polymerases and replication proteins. The E1 and E2 proteins bind to the origin of viral replication. The E1 protein is a helicase, the only viral-coded enzyme. Helicase unwinds the parental DNA strands in advance of replication. E2 protein forms a complex with the E1 protein and enables high-affinity binding to the origin of DNA replication. The E2 protein also participates in the segregation of viral DNA during cell division by binding the viral genome to
the cellular mitotic chromosomes. Other major functions of E2 protein include regulation of transcription of the viral genome: transcription of E6 and E7 viral genes is regulated and can be blocked by E2 protein. In addition, E2 protein may induce apoptosis (cell death) and therefore may function as a tumor suppressor protein.

E4 protein
The product of the E4 gene plays an important role in facilitating viral maturation and the release of viral particles from infected cells.

E5 protein
The E5 gene product induces an increase in the activity of a cellular mitogen-activated protein kinase. This enzyme enhances the cellular response to growth and differentiation factors resulting in continuous cellular proliferation. The effect of E5 protein on cell growth categorizes this protein as one of HPV oncoproteins (proteins that contribute to uncontrolled cell growth).

E6 and E7 proteins
The E6 and E7 proteins from low-risk HPV genotypes have been studied less extensively than those from high-risk HPVs. Apparently, E6 and E7 proteins from low-risk HPV genotypes play a major role in the viral life cycle. The E6 and E7 proteins from high-risk HPV genotypes are oncoproteins because their activity contributes to continuous cell proliferation and may lead to cell transformation. These oncoproteins interfere with the regulation of the host cell growth cycle by binding to cell proteins that regulate cell growth. The cellular regulatory proteins are the cell cyclins, the cyclin-dependent enzymes (kinases), and the so-called tumor suppressor proteins. Two tumor suppressor proteins play a major role in the regulation of cell growth: p53 and retinoblastoma protein pRB. The HPV E6 protein binds to the cell protein p53 and targets it for rapid degradation. HPV E7 protein binds to the protein RB, the retinoblastoma gene product. The outcome of this binding is stimulation of cellular DNA synthesis and continuous cellular proliferation. These events facilitate viral reproduction. The E6 protein, in addition to binding to the tumor suppressor protein p53, maintains a stably replicating viral genome within the nuclei of infected cells.

Viral replication
Studies of HPV life cycle had been hindered by the inability of these viruses to achieve high levels of replication under laboratory conditions. Some aspects of the viral life cycle have been studied in vitro and in various specialized epithelial cell systems that resemble the tissue architecture of normal host cells. Current advances in cell culture technology has made it possible to grow HPV in cultures, using differentiated skin cells. Productive viral infection in a natural setting takes place in stratified squamous epithelium of the skin or the mucous membranes. The epithelial cell layer is being continually replenished by the replication of basal cells at the basement membrane.

The first step in virus replication is viral binding to specific host cell receptors and viral entry into the host cell. Studies with HPV 16 demonstrate that virus-cell binding occurs via heparin sulfate proteoglycan cell receptors located on the epithelial cell surface or on the basement membrane. Binding does not initiate endocytosis. Separate cell receptors are involved in viral entry into epithelial cells. Viral genome replication takes place in the cell nuclei of infected basal cells but progeny virus is not produced. Productive infection must take place in differentiating cells that have migrated away from the basal layer. These cells remain active in the cell cycle due
to activity of the E7 protein. Transcription of late genes and synthesis of capsid proteins takes place in highly differentiated cells of the superficial layers of the epithelium. Newly synthesized L1 and L2 proteins are transported into the cell nucleus where they assemble into virus particles and package replicated viral DNA. Mature virus particles are released from host cells without cell lysis. (4)

PAPILLOMAVIRUSES AND HUMAN DISEASE
Clinical conditions associated with HPV

HPV infections range from mild skin lesions to malignancies. The majority of HPV infections are benign. Historically, HPV was first recognized as the cause of warts on the hands and feet. Warts are areas of hypertrophied skin filled with keratin, and generally resolve spontaneously within one to five years. Numerous HPV genotypes are known to cause warts (Table 1). Heck’s disease, an HPV infection of the oral cavity associated with HPV types 13 and 32 also tends to regress spontaneously. Epidermodysplasia verruciformis is a rare genetic disease characterized by warts on the trunk and arms. It can develop into squamous cell carcinoma. Recurrent respiratory papillomatosis of the larynx is primarily a disease of very young children.

Over forty distinct HPV types can infect the genital tract. The majority of these infections are asymptomatic and resolve spontaneously within two years.

Infections with multiple HPV types are frequently observed. In genital HPV infections the presence of multiple genotypes may tend to increase the severity of cervical disease.

<table>
<thead>
<tr>
<th>Disease</th>
<th>HPV type</th>
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<tbody>
<tr>
<td>Plantar warts</td>
<td>1, 2, 4, 63</td>
</tr>
<tr>
<td>Common warts</td>
<td>1, 2, 7, 4, 26, 27, 29, 41, 57, 65, 77, 3, 4, 10, 28</td>
</tr>
<tr>
<td>Flat warts</td>
<td>3, 10, 26, 27, 28, 38, 41, 49, 75, 76</td>
</tr>
<tr>
<td>Miscellaneous cutaneous lesions</td>
<td>6, 11, 16, 30, 33, 36, 37, 38, 41, 48, 60, 72, 73</td>
</tr>
<tr>
<td>Epidermodysplasia verruciformis</td>
<td>2, 3, 10, 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 50</td>
</tr>
<tr>
<td>Recurrent respiratory papillomatosis</td>
<td>6, 11</td>
</tr>
<tr>
<td>Heck’s disease</td>
<td>13, 32</td>
</tr>
<tr>
<td>Conjunctival papillomas/carcinomas</td>
<td>6, 11, 16</td>
</tr>
<tr>
<td>Genital warts</td>
<td>6, 11, 30, 42, 43, 45, 51, 54, 55, 70</td>
</tr>
<tr>
<td>Cervical intraepithelial neoplasia</td>
<td>More than 30 HPV types, many of which are high-risk</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>16, 18, 31, 45, 33, 35, 39, 51, 52, 56, 58, 66, 68, 70</td>
</tr>
</tbody>
</table>

Recently HPV oncoproteins E6 and E7 have been identified in some patients with oropharyngeal squamous-cell carcinoma. The overall survival of HPV-positive patients was substantially better than that of patients with HPV-negative oropharyngeal squamous-cell carcinoma.

Transmission and pathogenesis of HPV infection

Infection with HPV is extremely common. Genital infection with HPV is considered one of the most common sexually-transmitted diseases.

Transmission of HPV takes place, primarily, by skin-to-skin contact. Transmission may also occur through fomites, as in prolonged exposure to shared contaminated clothing, since HPV is highly resistant to heat and desiccation. Infection occurs through minute abrasions in the
epithelial cell layer. These abrasions expose the cells in the basal layer to viral entry. Cellular receptors, such as heparan sulfate, mediate the initial attachment of virus to cells, but the nature of specific receptors for viral entry is not precisely known. Basal layer cells have stem cell-like properties and are continually dividing. This provides a reservoir of cells for the suprabasal cell region that lies above the basal cell layer. It should be noted that excessive proliferation of basal cells is considered a feature of pre-malignant or malignant disease. Although the replication of the HPV genome is initiated in the cells of the basal layer, mature virus particles are generated in the differentiated keratinocytes of the suprabasal region of the epithelium.

In HPV infections that manifest as warts or condylomata, viral replication is associated with proliferation of all epidermal layers except the basal layer. Excessive multiplication of keratinocytes produces changes in cellular architecture, creating the typical papillomatous (wart-like) structures.

In genital HPV infection the clinical symptoms may be genital warts or a low-grade lesion called dysplasia (cervical intraepithelial neoplasia, grade I). Such lesions show altered patterns of cellular differentiation. Dysplasia is often cleared by the immune system in less than a year. The cellular immune system is apparently involved in the resolution of these lesions, but the precise mechanism of clearance is not yet understood. Those dysplasias that are not cleared may persist for several decades. Persistence of an infection with a high-risk HPV genotype is the major risk factor for the development of genital malignancies, such as squamous cell carcinoma or adenocarcinoma of the cervix. During carcinogenic progression of cervical lesions, HPV DNA may become integrated into the cellular chromosomes. This may disrupt the E1 and E2 genes, preventing vegetative viral replication and stimulating cell growth.

Factors involved in progression to malignancy

Although HPV had been demonstrated in all cases of cervical cancer, only a very small number of infected women will develop malignant disease, typically many years after the initial infection. Apparently factors other than HPV infection play an important role in cancer development.

Host-related factors

- **Age and sexual activity:**
  The highest risk of genital HPV infection coincides with the greatest cellular growth and differentiation in the cervical region. Increased cellular differentiation occurs at puberty and at first pregnancy and declines after menopause. Genital HPV infection is most common in women between 18 and 30 years of age, while cervical cancer is more common in women older than age 35, suggesting a slow progression of HPV infection to malignancy. Sexual exposure to multiple HPV genotypes introduces an additional risk factor.

- **Immune suppression**
  Cellular immunity is a major factor in controlling and eliminating an on-going HPV infection. Impairment of the immune system by co-infection with the human immunodeficiency virus or by immunosuppressive drugs limits the ability of the infected host to control the infection.

- **Cigarette smoking**
Local immune suppression induced by smoking and the mutagenic activity of cigarette components may contribute to persistence of HPV or to progression of infection to malignancy. Smoking is an important risk factor for higher grades of cervical disease.

- **Multiple pregnancies**
  This is a significant independent risk factor for moderate to high-grade cervical disease.

- **Genetic predisposition**
  The genetic make-up of the patient is an important factor in the development of cervical cancer: it may affect the susceptibility to HPV infection, the ability to clear the infection, or the rate of disease progression.

- **Co-infection with adeno-associated virus**
  Infection with the adeno-associated virus actually reduces the risk of cervical cancer. Apparently, a viral replication protein, Rep 78, interferes with the transcription of HPV 16 E6 and E7 oncogenes.

**Viral factors that contribute to tumor development**

- **High-risk genotype and viral persistence**
  The majority of human papillomaviruses are in the low-risk category and produce localized warts that do not undergo malignant progression even if left untreated. By contrast, infection with high-risk HPVs may lead to cancer. This is particularly true for specific high-risk genotypes such as HPV 16 and HPV 18. These viruses are responsible for slightly over 70% of cervical cancer cases.
  The high and low-risk HPV genotypes differ in a number of characteristics:
    - **Cell transformation**
      Some high-risk HPV genotypes are capable of inducing cellular transformation in transgenic mouse model systems and in cell cultures. This ability to transform cells has not been demonstrated for low-risk HPV genotypes.
    - **Integration of viral genome**
      One of the key events in cell transformation and carcinogenesis induced by high-risk HPV genotypes is the integration of the viral genome into the host chromosome. Such integration results in long-term persistence of viral DNA within the host cell and affects the expression of cellular genes. Cells with integrated viral genomes have a selective advantage over cells in which viral DNA is not integrated. Viral genes are partially expressed: E6 and E7 genes are expressed while other portions of the viral DNA are deleted or their expression is disturbed. Notably, the E2 gene transcriptional repressor is not expressed. The loss of E2 function may be critical for malignant progression since the E2 protein can block the transcription of E6 and E7 genes. Integration of viral genome into the cellular chromosome has not been shown for low-risk HPV genotypes.
    - **Viral oncoproteins**
      The E6 and E7 oncoproteins of high-risk HPV genotypes play a major role in cell transformation and in the induction of malignancy: these proteins bind cellular tumor repressor proteins involved in the regulation of the cell growth cycle. Weak binding between E6 and E7 gene products and the cellular regulatory proteins has been shown for the low-risk HPV genotypes.
o Induction of telomerase
The expression of the enzyme telomerase is essential for continuous cellular replication. The E6 and E7 oncoproteins of high-risk HPVs are able to induce the expression of telomerase. The activity of this enzyme facilitates cellular proliferation, extends the cellular life span and promotes cell immortalization. Telomerase induction has not been shown for low-risk HPV genotypes.

o Chromosomal abnormalities
Fully transformed cells generally present chromosomal abnormalities. The E6 and E7 oncoproteins from high-risk HPVs can induce genomic instability in normal human cells by generating mitotic defects through induction of centrosome abnormalities. In contrast, E6 and E7 proteins from low-risk HPVs are not capable of inducing centrosome abnormalities.

• Genomic variation
Human papillomaviruses may differ in certain biological and chemical properties and in pathogenicity due to genomic variation. For example, 5 different phylogenetic clusters have been described for HPV 16: European, Asian, Asian-American, African-1, and African-2. The oncogenicity of HPV variants may vary geographically and according to the ethnic origins of the population. An illustration of the effect of HPV variants on oncogenicity is provided by a large clinical study of 10,000 women in Costa Rica. In this study the European HPV 16 prototype and three variants were seen. The most common variant contained a single point mutation and was not associated with progressive disease. The second variant with a single mutation at a different location was associated with some high-grade squamous intraepithelial lesions. The third variant presented multiple mutations and was associated with malignancies.

• L1 gene mutation
Mutations within the HPV 16 L1 gene have been described in viral isolates from cervical cancer. These mutations result in an assembly-defective L1 capsid protein that cannot activate the antigen-presenting dendritic cells and induce an immune response. Indeed, only half of cervical cancer patients have been shown to generate antibody to the viral capsid protein.

• Immune evasion and viral oncoproteins
HPV oncoproteins may directly interfere with the host’s anti-viral immune defense mechanisms. The effects of HPV on the host’s immune system include depletion of intraepithelial Langerhans cells, which are essential for T cell priming, interference with activation of CD4 lymphocytes, (E7), downregulation of antigen presentation to T cells by the major histocompatibility complex (MHC), and interference with type 1 interferon signaling. These events result in lack of local inflammation, poor immune response to the virus, and viral persistence.

DIAGNOSIS AND TREATMENT
Diagnosis of HPV infections
A number of methods are currently available for determining the presence of HPV in clinical samples and for identifying the viral genotype:
Detection of viral nucleic acid:

- **In situ** hybridization
  Viral DNA can be identified in biopsy specimens by *in situ* hybridization with probes labeled with either radioisotopes or chemically reactive compounds. Reactions are detected by autoradiography, fluorescence, or by the development of a color reaction. Hybridization may be combined with amplification of nucleic acid by polymerase chain reaction (PCR).

- **Liquid hybridization assay**
  A Hybrid Capture assay kit is available for detection of HPV DNA in cervical samples. This is an immunoassay that uses chemiluminescence to detect the hybridization reaction between specimen target DNA and the RNA probe.

- **Immunohistochemical detection of DNA in biopsy tissue sections**
  An automated system (Gen Point) is available that detects as few as one or two HPV copies. Non-amplified immunoenzymatic assays detect 10 to 15 HPV copies.

- **General primer PCR assays**
  These assays use primers to amplify a broad spectrum of HPV types in a single PCR amplification. The primers target regions of HPV genome such as the L1 capsid gene. Various methods can be used to identify HPV genotypes after amplification. These methods include sequence analysis, restriction fragment length polymorphism, hybridization with type-specific probes, or enzyme-linked immunoassays using a mixture of HPV-specific probes.

- **Type-specific PCR assays**
  These assays are generally based on the sequence variation in E6 and E7 genes. The sensitivity of these assays is between 10 and 200 HPV copies per sample, depending on HPV type. Multiple PCR amplifications are required for each sample.
  A new HPV genotype assay based on proprietary Templex technology has been introduced recently. This assay detects and identifies 25 common HPV genotypes in a single-tube reaction using type-specific primers for E6 and E7 genes. The Templex assay provides semiquantitative information on each type when multiple HPV types are present in one reaction. The procedure can be completed within 5 hours.

- **Linear Array (Roche) genotyping test** is a reverse line blot assay for detection and genotyping of 37 types of HPV.

- **GenoFlow** is a recently introduced HPV array test that is capable of genotyping 33 types of HPV.

- **BD Onclarity HPV Assay** is a new polymerase chain reaction-based HPV screening test that targets the E6 and E7 DNA regions of the HPV genome. This assay can provide individual genotyping information for 6 HPV types while simultaneously screening for all 14 high risk virus types.

- **The Roche cobas HPV test** is an assay that allows HPV 16 and 18 genotyping concurrently with detecting 12 other high risk HPV genotypes. The test utilizes amplification of DNA by PCR as well as hybridization of 14 high risk HPV types in a single analysis.
• The H13 Assay, Hybribio Hong Kong (http://hybribio.com/), is an inexpensive PCR assay for the detection of 13 carcinogenic HPV types. This assay is useful for primary screening in settings with limited resources.

• Detection of HPV mRNA
   An assay is commercially available that detects mRNA of E6 and E7 genes. This assay can be done directly on Papanicolaou slides (Pap smears) and visualized using a fluorescence microscope.

• A recently introduced Aptima HPV Assay is an RNA test for a pool of 14 high risk HPV genotypes.

HPV cervical disease: diagnostic methods

CYTOLOGY
   Pap smear: Abnormal cells in the cervix can be detected through the use of the Pap smear. This test is used routinely in health screening programs in developed countries and less frequently in other areas of the world. In the United States the ability to detect abnormal cells in the cervix early in the disease process has cut the mortality from cervical cancer by 50%. In developing countries cervical cancer remains the most common cancer in women.

   The Pap smear has its limitations. These include inadequate samples and false negative results. False negative rates as high as 20% to 30% have been reported. Human error is probably a major factor: an average Pap smear slide has 50,000 to 300,000 cells and few abnormal cells that may be present can be easily missed. New methods of collection and processing of specimens for Pap smears have recently been developed to help reduce the number of false-negative results.

   If the Pap smear is abnormal, patients can be evaluated using instrumentation (colposcopy) and by cervical biopsy. In addition, HPV nucleic acid can be demonstrated in biopsy tissues by in situ hybridization with labeled probes.

   Recent studies have shown that tests for high risk HPV nucleic acid are more sensitive than cytology in screening for cervical cancer.

Treatment of HPV infection

• Therapeutic vaccination:
   This is a potential treatment method to clear existing HPV infections. Vaccines are targeted to stimulate cell-mediated responses in order to destroy infected cells. Some vaccines are currently in clinical trials.

• Treatment of skin warts:
   Warts generally do not require treatment and will regress spontaneously. Topical application of salicylic acid is available for home treatment. Other types of treatment include freezing the affected area (liquid nitrogen therapy), minor surgery, laser surgery, or chemical treatment with cantharidin (an extract from beetles).

• Treatment of anogenital warts:
   Treatment options include ablation, excision, or application of topical agents such as Polyphenol E ointment, Podofilox (active ingredient podophyllin) or imiquimod. Interferon-alpha has also been approved for the treatment of genital warts.

• Treatment of HPV cervical disease:
The majority of HPV-induced cervical dysplasias are transient. Most regress spontaneously within 12 to 36 months, as the immune system eliminates the virus. The treatment of lesions that persist depends on the size, stage, and histologic features of the lesion and the presence of lymph node involvement. Treatment may range from superficial procedures such as cryotherapy or laser therapy, excision of microinvasive cancers, to radiotherapy and hysterectomy. In 2014 the Federal Drug Administration (FDA) approved an anti-angiogenesis drug, bevacizumab, for treatment of advanced cervical cancer.

**PREVENTION: PAPILLOMAVIRUS VACCINE (5)**

The development of an HPV vaccine is of major public health importance. It is estimated that in the United States more than 6 million people become infected with HPV every year and nearly 10,000 women are diagnosed with cervical cancer. The mortality rate in the United States from cervical cancer is approximately 35%. Worldwide this disease is much more deadly, since 80% of cervical cancer cases occur in developing countries. Approximately 270,000 to 300,000 women die from cervical cancer each year worldwide. To a great extent the high mortality rate is due to the lack of widespread health screening and to a delayed diagnosis of this disease. It is estimated that the use of HPV vaccine will reduce cervical cancer mortality rate by 5% to 10% per year.

An additional benefit of the HPV vaccine would be its effect on fertility: *in vitro* fertility treatment is generally successful in 57% of uninfected women but only in 23% of HPV-infected women.

**Vaccine development:**

Preliminary pre-clinical research on the HPV vaccine was done by laboratories in the nonprofit sector. Theses studies demonstrated that L1, the major structural protein of HPV, has an intrinsic ability to self-assemble into virus-like particles (VLPs) when the L1 gene is expressed in host cells. The L1 protein contains the immunodominant neutralization antigens of the virus and is able to induce high levels of type-specific neutralizing antibody. Experimental studies with animal papillomaviruses showed that vaccination with L1 VLPs protected animals from high-dose challenge with homologous virus. The protection could be transferred passively with immunoglobulin G.

Commercial versions of HPV vaccine were developed by two companies, Merck and GlaxoSmithKline. Vaccines developed by both companies are subunit viral-like particle vaccines, (VLPs), composed of L1 protein, which is the major HPV capsid protein. L1 VLPs are deficient in their ability to package viral DNA. The presence of capsid protein L2 is required for DNA packaging and for production of infective virions. Merck’s HPV vaccine, Gardasil, has 4 HPV genotypes: HPV 16 and HPV 18, which are the primary cause of approximately 70% of all cervical cancers, and HPV 6 and HPV 11, which account for nearly 90% of external genital warts. The vaccine offers type-specific protection. The VLP particles for this vaccine are produced in yeast cells. Vaccine preparations also contain an alum adjuvant to maximize the immune response. The vaccine is administered intramuscularly in three doses over a six-month period. Gardasil was licensed in the United States in 2006 and approved for use in 9 to 26 year-old women. Gardasil is also approved for use in many European Union countries and in
Australia, Brazil, Canada, Mexico, New Zealand, and many others. In 2009 Gardasil was approved by the Federal Drug Administration for use in males aged 9-26 years in order to offer protection against genital warts. A new vaccine known as Gardasil 9 was recently introduced by Merck and approved by the FDA. This vaccine offers protection against 9 HPV genotypes (16, 18, 31, 33, 45, 52, 58, 6, 11). It prevents 90% of genital warts and cervical, vulvar, vaginal, and anal HPV infections. The vaccine is routinely started at 11 to 12 years of age and may be given to both males and females in 2 doses within a six months period.

The vaccine developed by GlaxoSmithKline is called Cervarix. This vaccine is bivalent, consisting of HPV 16 and HPV 18 L1 virus-like particles with a proprietary adjuvant ASO4 (alum and monophosphoryl). L1 particles are produced in insect cells using a recombinant insect virus, a baculovirus. The approval for use of Cervarix in the United States was granted in 2009. The vaccine is licensed for use in females 10 to 25 years of age. There is some evidence that Cervarix offers protection against pre-cancerous lesions associated with infection by HPV types 45, 33, and 31. The cross-protective effect of Cervarix is higher than that reported for the Gardasil vaccine.

Both Gardasil and Cervarix are highly immunogenic inducing a much greater antibody response than found after natural HPV infection. The peak antibody levels are reached 2 to 6 months after immunization. Antibody level decreases during the first 2 years after immunization reaching a plateau that is maintained for at least 4 to 5 years or longer. The anti-viral protection is type-specific. Protection against HPV infection can be detected as early as 1 month from the first immunization. Vaccinated women show a reduction in type-specific persistent HPV infection. The protection is in effect against both benign and malignant disease induced by HPV infection but neither vaccine alters the course of pre-existing HPV16/18 infections.

Even though the Gardasil and Cervarix vaccines appear to be highly effective there are a number of issues that still need to be explored. These issues include:

- Long-term safety of HPV vaccine
- Length of protection offered by vaccination
- Cost of vaccine and the availability of vaccine in poor countries, which have the greatest need for HPV vaccination.
- Cross-protection against HPV types not included in the vaccine
- Therapeutic effect of the vaccine against established infection
- Protective effect of the vaccine in men
- Effect of vaccination on the natural history of HPV infection
- Population groups to be vaccinated

SUMMARY

Papillomaviruses are widely distributed in the environment. They infect humans and other animal species and are highly species specific.

Most infections caused by papillomaviruses are benign and resolve spontaneously. Under certain conditions the infections do not resolve and progress to malignancy. The role of papillomaviruses in human disease had been difficult to demonstrate because these viruses grow poorly under laboratory conditions. The introduction of molecular diagnostic techniques
facilitated the demonstration of papillomaviruses in clinical lesions and the identification of these viruses as etiological agents responsible for human infections.

A variety of clinical conditions are caused by human papillomaviruses. These include skin and genital warts, miscellaneous cutaneous lesions, respiratory and conjunctival papillomas, cervical dysplasias, and cervical carcinoma. The clinical outcome of these diseases depends on various host factors and on the genotype of the infecting virus. The high-risk HPV genotypes promote the progression of HPV infection to malignancy. Other virus-related factors that facilitate development of malignant lesions are genetic variation among HPV types and immune evasion mechanisms.

Diagnosis of HPV infections is based on clinical symptoms and on the demonstration of HPV in clinical lesions. Identification of HPV relies primarily on molecular methods since cultivation of HPV in cell culture is difficult. PCR is used to amplify viral nucleic acid. A number of molecular methods are in current use and some commercial assays are available.

Recent introduction of HPV vaccine is of major public health importance. Two commercial companies, Merck and GlaxoSmithKline, have developed vaccines that protect against infections with selected HPV types. The Merck vaccine, Gardasil, contains 4 HPV genotypes: HPV 16 and HPV 18 associated with over 70% of cervical cancers, and HPV 6 and HPV 11, which cause most external genital warts. The vaccine is licensed in the United States and approved for use in 9 to 26 year-old girls and women. Recently Merck introduced a new vaccine, Gardasil 9, which offers protection against 9 HPV genotypes. The vaccine Cervarix, developed by GlaxoSmithKline, is also approved for use in the United States and in many other countries. This vaccine is bivalent, containing HPV types 16 and 18. The vaccines appear to be highly effective in protection against HPV infections and against pre-malignant disease associated with these infections.

REFERENCES
REVIEW QUESTIONS
DL-979
Select the one best answer:

1. Human papillomaviruses:
   a. infect the gastrointestinal tract exclusively
   b. infect cats and dogs
   c. infect the mucosal and cutaneous epithelium
   d. are not infectious

2. Human papillomaviruses
   a. cause infections that always progress to cancer
   b. cause infections that are generally benign
   c. infect only persons over 65 years of age
   d. are the primary cause of respiratory disease

3. Some characteristics of human papillomaviruses (HPVs) include:
   a. HPVs all belong to the same genotype
   b. HPVs do not cause cell transformation
   c. HPVs are subdivided into high-risk and low-risk categories
   d. HPV genome codes over 200 proteins

4. The human papillomaviruses:
   a. contain 2 capsid proteins
   b. have genome that consists of RNA complexed with lipids
   c. code all proteins needed for viral replication
   d. replicate in the cytoplasm of infected cells

5. The viral capsid:
   a. consists of proteins coded by “early” genes
   b. has a helical shape
   c. has an icosahedral shape
   d. consists of a single protein

6. Some characteristics of HPV proteins are:
   a. The E1 and E2 proteins are known as oncoproteins
   b. The E6 and E7 proteins bind to the cell tumor suppressor proteins
   c. The “early” proteins are the major structural proteins of HPV
   d. The function of “late” proteins is not known
7. Some characteristics of HPV replication and cultivation include the following:
   a. viral replication takes place in squamous epithelial cells
   b. HPV is easy to grow in standard cell cultures
   c. HPV can be cultured on special bacteriological media
   d. HPV will only grow in red blood cells

8. HPV’s are known to cause:
   a. gastrointestinal disease
   b. colds
   c. Roseola
   d. genital warts

9. HPV infection is transmitted by:
   a. contact with domestic animals
   b. the respiratory route
   c. the oral-fecal route
   d. skin-to-skin contact

10. HPV infections have some of the following features:
    a. Infection may resolve spontaneously due to clearance by the immune system
    b. Infection always progresses to malignancy
    c. Cervical cancer develops very rapidly after initial HPV infection
    d. Course of infection is not affected by viral genotype

11. Host factors that affect the progression of HPV infection to malignancy include:
    a. obesity
    b. consumption of coffee
    c. cigarette smoking
    d. vitamin deficiency

12. Viral oncoproteins may interfere with the host’s immune response by:
    a. inactivating macrophages
    b. binding to antibody molecules
    c. interfering with antigen presentation to cytotoxic T cells
    d. lysing T cells

13. Diagnosis of HPV infection is based on:
    a. cultivation of tissue samples in cell cultures
    b. cultivation of tissue samples in broth
    c. molecular techniques
    d. animal inoculation
14. HPV DNA in biopsy specimens:
   a. cannot be demonstrated by currently available methods
   b. can be amplified by PCR and demonstrated by molecular techniques
   c. can be demonstrated by hematoxylin stain
   d. can be demonstrated by Gram stain

15. The Templex HPV genotype assay:
   a. identifies multiple HPV types in a single-tube reaction
   b. is based on identification of HPV capsid proteins
   c. can only identify a single HPV genotype
   d. relies on cell culture for identification of HPV types

16. HPV vaccines, Gardasil and Cervarix:
   a. contain the L1 and the L2 viral proteins
   b. are produced in yeast cells
   c. are subunit viral-like particle vaccines composed of a single protein
   d. are poorly immunogenic

17. The HPV vaccines:
   a. are not able to prevent HPV infections
   b. prevent some HPV infections and pre-malignant lesions
   c. can be used as therapeutic agents to cure warts
   d. do not contain any high-risk HPV genotypes

18. Skin warts may be treated with:
   a. Pap smear
   b. adjuvants
   c. application of topical agents
   d. warm baths

19. Cervarix vaccine:
   a. is produced in insect cells
   b. contains 4 HPV genotypes
   c. does not contain any high-risk HPV types
   d. is licensed in the United States for use in men only

20. The original Gardasil vaccine contains:
   a. ten high-risk HPV genotypes
   b. live HPV viral particles
   c. a single HPV genotype
   d. four HPV genotypes