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*Review Article:*

# Molecular Diagnosis of Human Papilloma Virus Infection

AHMED ABOULNASR, M.D.*; NADINE SHERIF, M.D.*; MOHAMED ALI, M.Sc.*; MOHAMED ELMAHY, M.D.*; MOHAMED SHALABY, M.D.*; AMAL SOLIMAN, M.D.** and MOSTAFA SALEM, M.D.***

*The Departments of Obstetrics & Gynecology*, Clinical Pathology** and Pathology***, Faculty of Medicine, Cairo University*

## Abstract

Infection with human papillomaviruses (HPVs) can cause warts on cutaneous epithelium, while in the anogenital region these viruses can cause both genital warts and various forms of cancer in men and women. The main interest in HPV relates to its causative role in cervical cancer. Most HPV infections in young women resolve spontaneously, most frequently within a 24-month period.

Identification of HPV genotypes would require the use of type-specific probes in multiple in situ hybridization experiments. Alternatively, HPV-DNA can be directly isolated from clinical samples and detected by Southern blot or dot spot hybridization. However, such approaches are insensitive, labor intensive and unsuitable for high through put screening. Therefore, nucleic acid amplification methods have been developed to increase the sensitivity as well as the specificity of HPV-DNA detection.

***Key Words:*** *Human papilloma virus – Infection – PCR.*

## Introduction

**INFECTION** with human papillomaviruses (HPVs) can cause warts on cutaneous epithelium, while in the anogenital region these viruses can cause both genital warts and various forms of cancer in men and women. The main interest in HPV relates to its causative role in cervical cancer **[1]**. The development of cervical cancer is a multistep process, where HPV is necessary but an insufficient cause **[2]**.

Disease can only develop when there is persistent HPV infection of the cervical epithelium. Cervical cancer is a rare complication of infection with high risk HPV (HR-HPV), but every abnormal or dysplastic lesion of the cervix is potentially malignant and may develop into cervical cancer over time. Abnormal cervical epithelial cells can be detected microscopically following Papanicolaou (Pap) staining of conventional cervical smears or of the more homogeneous cell suspension from liquid cytology medium. This forms the basis of cervical screening programs for detection of women at risk of disease progression). Molecular detection of HPV provides a different approach to screening and patient management **[3]**.

The HPV virion has a double-stranded, circular DNA genome of approximately 7900bp, with eight overlapping open reading frames, comprising early (E), and late (L) genes and an untranslated long control region. The L 1 and L2 genes encode the major and minor capsid proteins. The capsid contains 72 pentamers of L1, and approximately 12 molecules of L2. The early genes regulate viral replication and some have transformation potential **[4]**.

At present, 118 HPV genotypes have been classified according to their biological niche, oncogenic potential and phylogenetic position **[5]** HPV isolate is described as a new genotype if the L 1 sequence differs by more than 10% from any previously known HPV genotype. Within a genotype, subtypes and variants can be distinguished, which differ 2-10% and maximally 2%, respectively. As intra and intergenomic recombination are rare, genotypes can be reliably classified by analysis of only part of the viral genome **[5]**.

The choice of a genomic region used for typing of viral isolates is important and must show enough discriminatory power for intertypic variation to distinguish a wide range of different genotypes, while intertypic variation, (i.e. heterogeneity within the same genotype) should be limited to permit

***Correspondence to:*** Dr. Mohamed Ali,
E-Mail: m.aly1918@gmail.com

reliable identification. HPV cannot be grown in conventional cell cultures, and serological assays have only limited accuracy [6].

As infection with HPV is followed by a humoral immune response against the major capsid protein [6], with antibodies remaining detectable for many years, serology is not suitable for distinguishing present and past infections. Consequently, accurate diagnosis of HPV infection relies on the detection of viral nucleic acid. Studies of HPV prevalence in various populations worldwide have shown a wide range of positivity rates [7]. In general, however, the prevalence of HPV is higher in young women compared to women over 30 years [8].

Most HPV infections in young women resolve spontaneously, most frequently within a 24-month period. The heterogeneous outcome of epidemiological studies may be due to several important factors. First, there appear to be marked differences in HPV prevalence in different populations with respect to age, frequency of cytological abnormalities and diversity of HPV genotypes. Secondly, multiple sampling and HPV-DNA detection techniques have been used, with different sensitivity and specificity, which may impact significantly on detection rates [9].

The natural history of HPV infection, including mode of transmission of the virus, development of persistent infection, clearance of the virus and interaction with the immune system is only partially known. At present, there is no established definition of a persistent HPV infection. One study suggested that women with mild or moderate dyskaryosis should only be referred for treatment after a persistent HPV infection of at least 6 months [10]. However, detection of HPV-DNA in consecutive samples should include genotyping or even analysis of molecular variants to confirm persistence of the same virus over time [11].

Detection of HPV-DNA and identification of HPV genotypes HPV-DNA can be detected by various methods, of which in situ hybridization. This method is based on the use of labeled probes that specifically hybridize to HPV-DNA. Although the sensitivity of this method is limited, it permits localization of HPV infection in the sample and possible co-localization with other markers [12]. Identification of HPV genotypes would require the use of type-specific probes in multiple in situ hybridization experiments. Alternatively, HPV-DNA can be directly isolated from clinical samples and detected by Southern blot or dot spot hybridization. However, such approaches are insensitive [13], labor intensive and unsuitable for high through put screening. Therefore, nucleic acid amplification methods have been developed to increase the sensitivity as well as the specificity of HPV-DNA detection.

*HPV nucleic acid detection:*

*1- Signal amplification systems:*

Signal amplification of ISH is possible using tyramide signal amplification [14], but the threshold remains low and consistent detection is difficult to achieve. Consequently, despite the interest of cytologists, the method has not been adopted for large scale HPV testing. The Hybrid Capture II system (hc2, Digene Corp., USA) is a non–radioactive signal amplification method based on the hybridization of the target HPV-DNA to labeled RNA probes in solution [15].

The resulting RNA-DNA hybrids are captured onto microtiter wells and are detected by a specific monoclonal antibody and a chemiluminescent substrate, providing a semi-quantitative measurement of HPV-DNA. Two different probe cocktails are used, one comprising probes for five low-risk genotypes 6, 11, 42, 43 and 44 and the other containing probes for 13 high-risk genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56,58, 59 and 68. This assay has become the standard in many countries, is widely used in clinical studies, and has FDA approval. However, hc2 has some limitations. It distinguishes between the high-risk and low-risk groups but does not permit identification of specific HPV genotypes.

The detection limit of approximately 5000 genome equivalents, makes it less sensitive than PCR [16] and cross-reactivity of the two probe cocktails [17] can reduce the clinical relevance of a positive result. Nevertheless, hc2 has been widely used in clinical trials worldwide and has been shown to be robust and reproducible as a screening assay [17]. Trials of the automated third generation Hybrid Capture assay were recently reported.

*2- Target amplification systems:*

PCR is the most widely used target amplification method, using a thermocycling process and employing oligonucleotide primers flanking the region of interest to amplify DNA in the presence of a thermostable DNA polymerase. Two approaches for detection of HPV-DNA by PCR are relevant.

*Type specific PCR versus broad-spectrum PCR:*

Type specific primers designed to amplify exclusively a single HPV genotype can be used, but to detect the presence of HPV-DNA in a single

sample, multiple type-specific PCR reactions must be performed separately. This method is labor-intensive, expensive and the type-specificity of each PCR primer set should be validated. Alternatively, consensus or general PCR primers can be used to amplify a broad spectrum of HPV genotypes. Such primers target a conserved region in different HPV genotypes. Since the L1 region is the most conserved part of the genome, several consensus PCR primer sets are aimed at this region **[18]**.

General primers in the E 1 region have also been described **[19]** and several other broad-spectrum PCR primers were reported but have not been extensively used in clinical situations. Three different designs of general PCR primers can achieve broad-spectrum detection of HPV DNA. The first incorporates one forward and one reverse primer aimed at a conserved region, but fully complements only one or a few HPV genotypes. To compensate for the mismatches with other HPV genotypes, the PCR is performed at a low annealing temperature. The GP5+/6+ PCR system is an example of this approach **[20]**.

The second class of general PCR primers comprises forward and reverse primers, which contain one or more degeneracies to compensate for the intertypic sequence variation at the priming sites. These primers do not have to be used at a lower annealing temperature. The MY09/11 is an example of a degenerated PCR primer set **[21]**.

In fact, this primer set comprises a complex mixture of many different oligonucleotides. The disadvantage of this design is that synthesis of oligonucleotides containing degeneracies is not highly reproducible and results in high batch-to-batch variation. Therefore, each novel batch of primers should be carefully evaluated to check the efficacy of amplification for each HPV genotype **[22]**.

The third option is to combine several distinct forward and reverse primers, aimed at the same position of the viral genome. These primers do not contain random degeneracies, but may contain inosine, which matches with any nucleotide. Using a defined mixture of non-degenerate primers has the advantage that the oligonucleotides can be synthesized with high reproducibility, and PCR is performed at optimal annealing temperatures. Examples of such primer sets are the PGMY primers **[22]** and the SPF 10 primers **[23]**.

Besides the choice of primers, the size of the PCR product is also important. In general, the efficiency of a PCR reaction decreases with increasing amplimer size. Subjecting clinical samples to treatments, such as formalin-fixation and paraffin-embedding, degrades DNA. Consequently, the efficiency of PCR primers generating a small product is considerably higher than primer sets yielding larger amplimers **[24]**.

*Real-time PCR:*

Real-time PCR can also be used to detect HPV-DNA. Type-specific PCR primers can be combined with fluorescent probes for real-time detection **[25]** although multiplexing several type specific primers within one reaction can be technically difficult. Broad-spectrum PCR primers have also been used in real-time PCR **[26]** but are less amenable to quantitation than a type specific primer system. Due to the sequence heterogeneity of different HPV genotypes, genotyping of PCR products from broad-spectrum PCR requires a mixture of probes and since these will all have different hybridization characteristics, standardization is difficult **[27]**.

*Reverse Transcriptase-PCR:*

It is also possible to look for specific viral RNA by incorporating a reverse transcriptase (RT) step before PCR amplification. Although most HPV detection strategies used for epidemiological studies and clinical management have, thus far, been DNA based, detection of expression of HPV oncogenes may have significant clinical value. For example, **[28]** developed a real-time RTPCR for HPV 16 and 18 E7 transcripts and suggested that it may be more specific for the detection of symptomatic infections.

Wang-Johanning et al. **[27]** also described an HPV16 E6/E7 quantitative real-time RT-PCR and found that expression increased coordinately with severity of the lesion. There is currently one commercially available RNA based HPV assay, the PreTect HPV Proofer (Norchip AS Klokkarstua, Norway). This assay incorporates NASBA amplification of E6/E7 mRNA transcripts prior to type specific detection via molecular beacons for HPVs 16, 18, 31, 33 and 45.

Initial data, on the prognostic value and specificity for underlying disease, is promising, but the clinical value of this method compared with DNA based assays remains to be determined in large-scale prospective studies. The physical state of the HPV genome has also been explored as a potential diagnostic marker. Integrated virus is associated with a neoplastic phenotype/high grade disease, where loss of the regulatory E2 protein on integration results in up-regulation of oncogenes E6 and E7 **[29]**.

Detection of integrated HPV can be performed by identification of viral cellular fusion transcripts such as the APOT technique [11] and by ligation mediated PCR [30] with detection of integrate-derived HPV transcripts showing a high specificity for high-grade disease and cancer. However, as application is currently restricted to identification of types 16 and 18, they are at present more appropriate for epidemiological studies.

3- *Detection and analysis of amplification products:*

PCR amplimers can be detected easily by standard agarose gel electrophoresis. However, subsequent sequence-specific analysis considerably increases both the sensitivity and specificity of the assay. Several methods have been developed for this purpose.

*PCR and restriction fragment length polymorphism (PCR-RFLP):*

After amplification, the sequence composition of a PCR product can be investigated by restriction enzymes. Digestion of PCR products with restriction endonucleases generates several fragments, which can be resolved by gel electrophoresis, yielding a banding pattern. This method is straight forward but labor-intensive. More importantly, the method depends on availability of restriction enzymes capable of detecting specific mutations. Consequently, detection of multiple HPV genotypes, present in different quantities in a clinical sample by PCR-RFLP is usually complex and the sensitivity to detect minority genotypes is limited [31].

*Hybridization analysis of PCR products:*

A common way to investigate the sequence of PCR products is hybridization with one or more oligonucleotide probes. Type-specific PCR products can be confirmed with corresponding type-specific probes. The original method is Southern blotting, where a PCR product is electrophoresed prior to transfer to a membrane that is subsequently hybridized to a labeled probe [13]. However, Southern blotting is labor-intensive and not suitable for routine application. Therefore, alternative hybridization formats have been developed.

*Microtiter plate hybridization:*

To increase the through put of a diagnostic assay, hybridizations to oligonucleotide probes can be performed in microtiter plates. Biotin labeling of one of the primers generates labeled PCR products that are then captured onto streptavidin-coated microtiter wells. Double-stranded DNA is denatured under alkaline conditions and the unattached strand is removed by washing. A labeled oligonucleotide probe is added, which hybridizes to the captured strand. Hybrids can be detected following binding of conjugate and substrate reaction [32].

The Roche Molecular Systems Amplicor HPV MWP assay was recently described. This method is based on the detection of 13 high-risk genotypes by a broad-spectrum PCR in the L 1 region, amplifying a fragment of approximately 170bp. The heterogeneous interprimer region is detected with a cocktail of probes for high-risk genotypes.

Preliminary data suggests this assay is more sensitive than hc2 for detection of the same HR-HPV types (21 st International Papillomavirus Conference, Mexico, February 2004), although further work is required in prospective cohorts to assess whether this increased sensitivity is a benefit. An advantage of this method is the high throughput of the microtiter format. Therefore, this method is suitable for distinguishing HPV-DNA positive and negative samples as a first step in HPV diagnosis.

*Direct sequence analysis of PCR products:*

Rapid sequencing methods of PCR products are also now available for high throughput, thus permitting application in routine clinical analysis [32]. However, sequence determination is not suitable when a clinical sample contains multiple HPV genotypes. Sequences, which represent a minority species in the total PCR product, may remain undetected. In turn this may underestimate the prevalence of infections with multiple HPV genotypes, with important consequences for vaccination or follow-up studies [33].

This was confirmed in a recent study comparing sequence analysis of SPF 10 PCR products with reverse hybridization in 166 HPV-positive cervical scrapes. Compatible HPV genotypes were found in all samples. Direct sequence analysis detected multiple types in only 2% of the samples, while reverse hybridization found multiple types in 25%. The presence of multiple HPV genotypes is a common phenomenon in many patient groups. Up to 35% of HPV-positive samples from patients with advanced cytological disorders and more than 50% of HIV-infected patients contain multiple HPV genotypes, whereas multiple genotypes are less prevalent in carcinoma patients [34].

*Reverse hybridization:*

Reverse hybridization provides an attractive tool for simultaneous hybridization of a PCR product to multiple oligonucleotide probes. This method comprises immobilization of multiple oligonucle-

otide probes on a solid phase and addition of the PCR product in the liquid phase. Hybridization is followed by a detection stage.

The most frequently used reverse hybridization technology comprises a membrane strip containing multiple probes immobilized as parallel lines, called line probe assay (LiPA); line blot assay (LBA) or linear array (LA). A PCR product is generated, usually using biotinylated primers. The double stranded PCR product is denatured under alkaline conditions and added to the strip in a hybridization buffer. After hybridization and stringent washing, the hybrids can be detected by addition of a streptavidin-conjugate and a substrate, generating color at the probe line, which can be visually interpreted. This method permits multiple HPV type detection in a single step and requires only a limited amount of PCR product. Reverse hybridization methods are particularly useful for the detection of type specific infections and multiple genotypes **[35]**.

*Screening and progression prognostic biomarkers technologies:*

Because molecular testing for HR-HPV DNA may detect infection too early in the process, with only a small subset of women developing disease that progresses to cancer, there is interest in defining secondary markers that have potential application in identification of women who need to be followed more closely because they are at higher risk of developing high-grade lesions, especially, when the positive predictive value of current screening strategies will be diminished in a vaccinated population **[36]**.

Then, the impetus for new screening or progression technologies in the developed world is thus predominately driven by the need to increase Molecular Diagnosis of Human Papillomavirus Infections positive predictive value and reduce overmanagement of low-grade and often transient abnormalities. In these situations, several surrogate markers are in research.

1- *HPV viral load:*

Several studies have suggested that a high HPV-DNA viral load may be a candidate marker that could help identify women at greater risk of CIN progression. It has been reported that average HPV DNA copy number increases significantly with the grade of CIN mainly for HPV 16, but not for other HR-HPV types.

Some studies have pointed out that high viral load in cytological normal epithelium could also be a risk factor for neoplastic progression but other studies suggested an important limitation to the utility in screening algorithms for the substantial overlap oh HPV load values between women without and with CIN and the common presence of more than one carcinogenic HPV type. Real-time PCR techniques have been developed to quantify HPV in clinical samples.

Moreover, the HCII provides semiquantitative measurement of HPV-DNA, and some studies have demonstrated that the estimated HCII load correlated well with the precise load generated by RT-PCR. However, real-time PCR assays more accurately measure HPV 16 viral load by adjusting the signal obtained for HPV 16 DNA with the amount of cellular DNA calculated for amplification of a human gene, therefore providing a more accurate viral load (33). However, due to low multiplicity for different HR-HPV types, real-time PCR methods are not suitable as a high-throughput screening tool.

*2- HPV mRNA:*

Although HR-HPV genotypes are associated with any grade of dysplasia, these types can be detected in a significant proportion of women with normal cytology. It is known that HPV E6 and E7 genes are overexpressed throughout the thickness of epithelial cells in high-grade lesions and cancer. Then, mRNA could be more efficient than cytology for the triage of HPV DNA-positive women, and provides high septicity for high grade cervical intraepithelial neoplasia identification **[30]**.

Some authors have developed a real time reverse transcriptase amplification (RT-PCR) for HPV detection strategies and suggested that it may be more specific for the detection of symptomatic infections and quantitative increased coordinately with severity of the lesion **[27]**.

*3- HPV integration (E2/E6-7 ratio):*

Most HR-HPV infections are either latent or permissive. Latent infections are not very well defined, but it is assumed that the viral genome is maintained as an episome in the basal and parabasal cells of the epithelium without inducing obvious phenotypic alterations in the host cell. The transformation process is characterized by the deregulation of viral oncogenes E6 and E7 in cycling cells which ultimately results in chromosomal instability and the accumulation of mutations. The underlying mechanisms for deregulation are manifold.

Integration of the HPV genome is a characteristic step in cervical carcinogenesis and its appear-

ance correlates with the progression of precancerous lesions (CIN2/3) to invasive carcinoma [22]. However, integration is not mandatory in this process and was shown to be HPV-type dependent. The loss of the viral E2 gene is a common consequence of HPV integration. This event may lead to an elevated expression of the oncogenes E6 and E7 since E2 is no longer able to repress the expression of the viral oncogenes [27].

Several investigators have also focused on the impact integration may have on the host genome. Methods for detection of integrated HPV have been described. However, they are affected by similar limitations described for HPV viral load. On the other hand, cervical epithelial cells for women with CIN may simultaneously contain episomal and integrated HPV DNA. Recent data suggest that integration frequency in CIN3 is variable by HPV genotype, further reducing the desired gains in specificity [31].

*Clinical utility of molecular HPV diagnosis:*

The development of highly sensitive DNA detection assays over the past years has revolutionized the diagnosis of HPV and allowed various crucial aspects of HPV infections to be studied. However, diagnostic test results should be interpreted with care and require careful laboratory validation [17]. There is a clear need for well characterized international quality control panels to compare the various diagnostic methods.

The implications of HPV-DNA detection for patient management need to be further assessed. Recent studies have shown that the prevalence of HPV-DNA and of multiple HPV genotypes in the same patient is higher than expected. Also, the efficacy of large community-based HPV screening studies depends on the accuracy and predictive values of the diagnostic assays used. To identify women with an increased risk for cervical neoplasia, it is clear that detection of HPV-DNA alone is insufficient and novel algorithms are being developed which combine cytological screening and HPV-DNA analysis, to optimize the positive and negative predictive values for development of disease [10].

Accurate HPV genotyping is essential for adequate classification of patients into low-risk or high-risk groups. Furthermore, preliminary evidence suggests that the presence of multiple HPV genotypes may reflect repeated exposure and may relate to increased risk for disease progression. However, this is controversial as the evidence is inconclusive [38].

HPV persistence also has been identified as an important risk factor and should be included in clinical testing algorithms. However, HPV infections can only be classified as truly persistent if identical subtypes are detected in consecutive samples during follow-up studies. HPV viral load may also be a valuable predictor of disease although currently accurate quantitative viral load measurements are technically difficult in clinical samples [39].

Recently, the results of a HPV 16 VLP-based vaccine trial indicated that the development of type-specific antiviral therapies or vaccines requires the introduction of suitable algorithms for detection and genotyping of HPV. These methods are also necessary for accurate follow up during clinical trials, monitoring of antiviral or surgical treatment as well as triage and management of patients. To address cervical cancer detection worldwide and assess the geographic distribution of HPV genotypes, extensive epidemiological studies are required. Given the substantial genetic heterogeneity of HPVs and the possible clinical relevance of specific subtypes, specific molecular tools will be required. Novel low- or high-density DNA probe arrays (DNA chips) may provide a useful technology for such studies [36].

It has been established that there is variation in interpretation of ASCUS Pap smears even among expert cytopathologists. In some women, ASCUS indicates real pathology and in others it represents only a vigorous reactive change that is benign. In the United States, about 2.5 million ASCUS Pap results are reported each year. A survey of U.S. laboratories found that a median of 2.9% of all Pap smears are reported as ASCUS, with 10% of laboratories reporting more than 9% ASCUS results [39].

Several strategies are currently in use to manage patients with ASCUS Pap smear results. Some clinicians repeat the Pap smear in 4 to 6 months. Many ASCUS patients directly undergo colposcopy to detect the 10 to 20% who prove to have an underlying higher-grade lesion (e.g., LSIL or HSIL). Identifying women at high risk by testing for HPV DNA avoids unnecessary colposcopy procedures. Patients with ASCUS who are positive for high-risk HPV DNA are referred for colposcopy. Those who are negative for HPV DNA undergo a repeat Pap smear at 6 months and 12 months. If these are also negative, the woman is returned to a routine screening schedule [40].

HPV DNA testing may reduce costs by triaging patients into appropriate management strategies and reducing unnecessary colposcopy and less frequent screening in low-risk patients. Computer-based mathematical models that incorporate a simulated natural history of HPV carcinogenesis have been used to assess the cost-effectiveness of HPV screening strategies **[40]**.

These studies showed small differences between ASC-US management strategies in terms of reducing the incidence of cervical cancer. However, there were considerable differences in costs associated with the management strategies. Immediate colposcopy was always more expensive than the other strategies. Reflex HPV DNA testing of a liquid-based cytology specimen or testing of a co-collected second specimen at the time of the initial Pap smear was less expensive than repeat cytology, partly because these strategies eliminate the need for an additional clinic visit and reduce the number of colposcopies by 40 to 60% **[41]**.

Stratifying ASC into the 2001 Bethesda System categories of ASC-US and ASC-H made little difference in terms of clinical benefit or costs **[42]**. In addition to improving the management of women with ASC, the superior negative predictive value of HPV DNA testing may allow longer screening intervals. The computer models showed that a biennial or triennial cervical cancer screening program involving liquid-based cytology and reflex HPV DNA testing is more effective and less costly than annual screening by conventional cytology for women with ASC **[43]**.

It is estimated that savings of more than $15 billion would be gained over the lifetime of a typical cohort of 18- to 24-year-old women by using biennial liquid-based cytology screening and reflex HPV DNA testing **[42]**. In addition, termination of screening at 75 years of age would capture 97.8% of the benefits of lifetime biennial screening and would be less expensive **[43]**.

Using HPV DNA screening alone as a primary biennial screen becomes more cost-effective than biennial Pap screening only if the cost per HPV DNA test is $5 or less **[43]**. Many insurance companies are supporting HPV DNA testing as an adjunct to Pap smear screening and are providing reimbursement for these tests.

In the United States, 50 to 60% of women diagnosed with invasive cervical cancer have not had a Pap smear within the preceding 3 to 5 years or have never had a Pap smear **[42]**. It is paramount to a clinically effective and cost-effective screening program that Pap smear testing and management of an abnormal result is consistently available to all women and that unscreened women are encouraged to use the Pap smear screening program.

Evidence-based consensus guidelines for the management of cervical cytological abnormalities and cervical cancer precursors were developed at the American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Conference in September 2001. The new Bethesda 2001 terminology for reporting cervical cytology results, fluid-based cytology methods, molecular methods for detecting high-risk HPV types, data from the ALTS trial, and cost analyses were all taken into consideration in development of the guidelines. **[43]**.

Recommended management of women with ASC-US includes three options.

(i) HPV DNA testing is the preferred approach if fluid-based cytology is used or if specimens are co-collected for HPV DNA testing. If HPV DNA testing is negative for high-risk HPV types, the patient undergoes repeat cytology testing at 12 months. For women whose test is positive for high-risk HPV types, referral to colposcopy is recommended. If biopsy confirms CIN, patients are treated as per standard practice for CIN. If biopsy does not confirm CIN, then (a) Pap smear should be repeated at 6 and 12 months with referral back to colposcopy if cytology results show ASC-US or greater or (b) HPV DNA testing should be repeated at 12 months with referral back to colposcopy if high-risk HPV types are found.

(ii) If a program of repeat cervical cytology is used, ASC-US patients should undergo Pap testing at 4- to 6-month intervals until two negative results are obtained. The patient can then be returned to routine cytologic screening. If any repeat Pap smear shows ASC-US or greater, referral to colposcopy is recommended.

(iii) When immediate colposcopy is used, women with biopsy-confirmed CIN are treated as per standard practice for CIN. If biopsy does not show CIN, patients undergo repeat Pap smear at 12 months.

In postmenopausal women with ASC-US and clinical or cytologic evidence of atrophy, a course of intravaginal estrogen is recommended if there are no contraindications to estrogen use. A Pap smear is performed about a week after completion of the estrogen regimen. If Pap smear cytology is negative, the test should be repeated in 4 to 6 months. If the repeat Pap smear shows ASC-US

or greater, the patient is referred to colposcopy. Immunosuppressed women with ASC-US should be directly referred to colposcopy. Pregnant women should be managed in the same manner as non-pregnant women **[42]**.

Patients with ASC-H Women with Pap smear results indicating ASC-H should be directly referred to colposcopy. If no lesions are identified by colposcopy, a review of the Pap smear, colposcopy, and histology results is recommended, if possible. If review yields a revised interpretation, management guidelines for the revised interpretation should be followed. If colposcopy confirms ASC-H, a Pap smear should be performed at 6 and 12 months with referral back to colposcopy if cytology results show ASC-US or greater. Alternatively, HPV DNA testing can be done at 12 months with referral back to colposcopy if high-risk HPV is found **[41]**.

Patients with LSIL Women with Pap smear results indicating LSIL should be directly referred to colposcopy. If colposcopy is satisfactory and fails to confirm CIN, a Pap smear should be performed at 6 and 12 months with referral back to colposcopy if cytology results show ASC-US or greater. Alternatively, HPV DNA testing can be done at 12 months with referral back to colposcopy if high-risk HPV is found. If colposcopy is unsatisfactory and fails to confirm CIN, management options include repeat cytologic testing 6 and 12 months with referral back to colposcopy if cytology results show ASC-US or greater or HPV DNA testing at 12 months with referral back to colposcopy if high-risk HPV is found **[43]**.

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# التشخيص الجزيئى لعدوى فيروس الورم الحليمى البشرى

فيروس الورم الحليمى البشرى يعد واحداً من أكثر الأسباب شيوعاً للأمراض المنقولة جنسياً فى كل من الرجال والنساء فى جميع أنحاء العالم وقد تم التعرف على أكثر من ٢٠٠ نوع من فيروس الورم الحليمى البشرى على أساس بيانات تسلسل الحمض النووى.

وبناء على ارتباطه بسرطان عنق الرحم فيروس الورم الحليمى البشرى يمكن أن يصنف إلى نوعين ومرتفع الخطورة ومنخفض الخطورة وتشمل أنواع فيروس الورم الحليمى البشرى المرتفعة الخطورة أنواع ١٦–١٨–٣١–٣٣–٣٤–٣٥–٣٩–٤٥–٥١–٥٢–٥٦–٥٨–٥٩–٦٦–٦٨–٧٠.

فيروسات الورم الحليمى البشرى التى تنتقل بالاتصال الجنسى تؤدى إلى واحدة من ثلاث نتائج محتملة:

- الثاليل الشرجية التناسلية.
- العدوى الكا منة الغير نشطة.
- عدوى نشطة.

قد يكون من الأفضل استخدام مسحة عنق الرحم مع تفاعل البلمرة المتسلسل لفيروس الورم الحليمى البشرى فى فحص السيدات ذوات أعناق الأرحام التى تبدو غير طبيعية وفى فحص سرطان عنق الرحم.