MEDWELL PUBLISHING
Research Journal of Biological Sciences 4 (1): 29-36, 2009
ISSN: 1815-8846

The Human Papillomaviruses (HPVs) and HPV DNA Testing

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Abstract: The fact that some viruses act as carcinogens has long since been known. Amongst these viruses are some genotypes of the Human Papillomaviruses (HPVs). HPV is most frequently associated with cervical cancer, that is, cancer of the cervix or neck of the uterus. In fact, 95-100% of all cervical cancers are caused by infection with HPV. HPV also causes a high proportion of other anogenital cancers. In 1995, the International Agency for Research on Cancer (IARC) concluded that HPV types 16 and 18 are carcinogenic to humans; HPV types 31 and 33 are probably carcinogenic to humans whilst some HPV types other than 16, 18, 31 and 33 are possibly carcinogenic to humans. This review focuses first on the structure, classification and genome of these particular viruses. Particular attention is given to those features that play a role in the carcinogenicity of particular HPV genotypes. Given the close association between HPV and cervical cancer, detecting the presence of HPV in a particular patient and more specifically, the presence of particular genotypes of HPV, may give an indication of the likelihood of progression to precancerous and cancerous changes in the cervix. In fact, there is much evidence that screening of women with both cytology and HPV DNA tests increases sensitivity for detection of Cervical Intraepithelial Neoplasia (CIN) 3 or cancer sufficiently to permit longer screening intervals than with cytology alone. However, it is important to realize that the presence of HPV does not mean that a woman has or will develop cervical disease. Thus, there is still a dilemma as regards the real utility of HPV DNA testing. Screening and diagnostic procedures for cervical cancer will be discussed in the second part of this review, with special emphasis on HPV DNA testing. The benefits of HPV DNA testing in specific situations will be highlighted, particularly in the case of a diagnosis of Atypical Squamous Cell of Undetermined Significance (ASCUS).

Key words: Structure, classification, genome, cytology, HPV DNA testing

INTRODUCTION

Structure: Human Papillomaviruses (HPVs) form icosahedral, non-enveloped particles with a diameter of approximately 55 nm. They contain a double-stranded, supercoiled and circular DNA genome of 7500-8000 base pairs (IARC, 1995), which weighs about 5×10^6 Da. Only one strand of their DNA is coding (Cubie, 2007). Papillomaviruses are resistant to organic solvents and to heating at 56°C. HPV particles have a buoyant density of 1.34 g mL^{-1} in caesium chloride and a sedimentation coefficient of 300.

The viral capsid is composed of 72 capsomeres, which are arranged on an icosahedral surface lattice. Sixty of the capsomeres arte 6-coordinated and the remaining 12 are 5-coordinated. Capsomeres consist of a trunk with proximal and distal thickening. They associate at their base and project radially. Each capsomere is made up of 5 identical subunits and therefore exhibits a 5-fold symmetry (Fig. 1) (IARC, 1995).

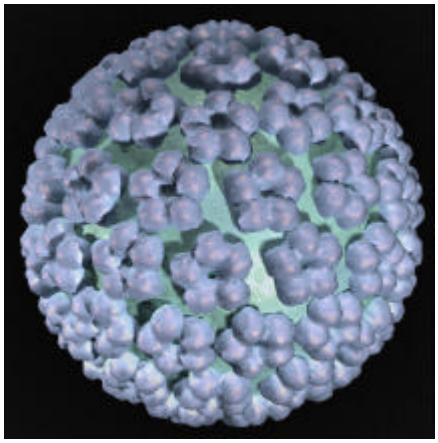

Fig. 1: Three-dimensional picture of HPV showing the capsomeres. Each capsomere is composed of 5 subunits and therefore exhibits a 5-fold symmetry. Source: http://www.med-ars.it/virus/hpv1. jpg

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Table 1: Classification of HPV according to their oncogenic risk. Adapted from: Palefsky (2002)

Risk category	HPV type	Oncogenic risk
Low	6, 11	Rarely found in cervical cancer-mostly cause genital warts.
Intermediate	33, 35, 39, 52, 52, 56, 58, 59, 68	Found in about 25% of cervical cancers. Rarer than the high-risk types.
High	16, 18, 31, 45	Found in about 75% of cervical cancers. Types 16 and 18 are the most common

Table 2: Classification of human papillomavirus by genus, according to the international council on taxonomy of viruses

Genus	Type	Associated lesions
"	HPV -2, -27, -57	Common skin warts, also frequently in genital warts in children
	HPV -18, -39, -45, -59	Mucosal lesions, high risk, more frequent in adenocarcinomas
	HPV -16, -31, -33, -35, -52, -58, -67	Mucosal lesions, high risk, more frequent in squamous carcinomas
	HPV-6, -11, -13, -44, 74	Benign mucosal lesions-genital warts, laryngeal papillomas
$	HPV-5, -8	Cutaneous benign and malignant lesions in immunosuppressed patients
(	HPV-4, -65	Cutaneous benign lesions
µ	HPV-1, -63	Cutaneous benign lesions, frequently on feet

Source: Cubie (2007)

Classification: The papillomaviruses are the viruses belonging to the Papillomaviridae family. Previously, they were classified as a subfamily together with the polyomaviruses and the vacuolating virus of rhesus monkeys as belonging to the Papovaviridae family. These 3 groups of viruses used to be classified together because of the similar morphology of their capsids and also because they all contain a double-stranded and circular deoxyribonucleic acid (DNA). However, the biology, mode of action, genomic size and organization and nucleotide and amino acid sequence of papillomaviruses are different from that of the other 2 species. This knowledge has resulted in papillomaviruses being classified as a separate family.

Papillomaviruses are found in birds, reptiles and in many mammalian species (including humans), but different types are specific to each species. They infect squamous epithelium and mucous membranes, causing benign warts and sometimes tumours. Different HPVs have evolved to fill different biological niches, with the particular tissues infected and the appearance of the lesions produced being associated with specific virus types (Cubie, 2007). These types are defined by genomic analysis and therefore represent genotypes. In fact, an HPV type is defined as a complete genome where the sequence of the L1 gene is at least 10% dissimilar that of any other type. Subtypes vary by 2-10% in the L1 sequence, whereas variants have >2% variation in L1.

HPV genotypes can also be classified as low, intermediate or high risk according to the extent of their oncogenic potential (Table 1). More than 200 HPV types exist and the genome of approximately 100 types has been fully sequenced. Table 2 shows the HPV classification according to the genus. Eight genera have been agreed and the majority of HPVs fall into the alpha genus (Cubie, 2007).

HPV-GENOMIC ORGANIZATION AND ROLES OF GENE PRODUCTS

The circular DNA genome of all papillomaviruses can be divided into 3 segments of unequal size-the upstream regulatory region (URR), the early (E) region and the late (L) region. The URR, also called the long control region (LCC) or the non-coding region (NCR), represents about 10% of the genome (IARC, 1995). It is involved in the initiation of viral replication and is important for the regulation of gene function (Cubie, 2007). The early region, which makes up about 50% of the viral genome (IARC, 1995), is composed of 2 large (E1 and E2) and several smaller (E4-E7) reading frames (Cubie, 2007). The E genes encode proteins with various regulatory functions. The late region, on the other hand, makes up 40% of the genome and is made up of 2 large genes (L1 and L2) that code for the capsid proteins (IARC, 1995) (Fig. 2).

The coding sequences are distributed in all open reading frames. Genes E4, E5 and E7 encode single polypeptide chains and each L gene encodes a single protein. Splicing events lead to the production of different proteins with different functions (Cubie, 2007). In fact, E1/, E2 and E6, give rise to >1 protein through differential splicing (IARC, 1995).

E1 and E2 genes and proteins: The E1 gene is important for episomal maintenance and is often disrupted by integration (Cubie, 2007). It is the largest open reading frame of papillomaviruses, being transcribed into polycistronic RNAs that start at the E6/E7 promoter. It encodes 2 known polypeptide products, the largest of which is needed for replication.

E2 genes of papillomaviruses encode proteins that differ in size and which have highly conserved N-terminal transcription activation domains of about 200 amino acids and highly conserved carboxyl-terminal DNA binding

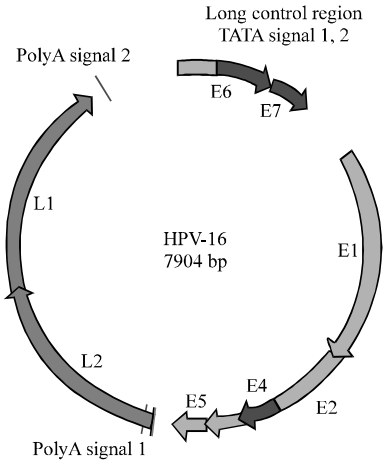

Fig. 2: The genome of HPV-16. Source: http://www.answers.com/topic/hpv-16-genome-organi-zation-png

domains of about 85 amino acids. These 2 domains are linked by sequence diverse hinge regions. E2 proteins form dimers, which function as DNA binding factors, their carboxyl terminal domains having high specificity for the sequence 5'-ACCGNNNNCGGT-3'. Bound to DNA (IARC, 1995) and together with E1 E2 proteins can function as transcription activators and repressors or as modulators of replication (Cubie, 2007).

E4 gene and proteins: E4 proteins are involved in maturation of virions and in their release from differentiated keratinocytes (Cubie, 2007). In fact, E4 proteins form structured filamentous cytoplasmic networks that co-localise with cytokeratin filaments. E4 proteins of different HPV's have little nucleotide sequence similarity yet they share a high proline content (IARC, 1995).

E5 gene and proteins: The E5 gene is not found in all papillomaviruses and is frequently lost or remains unexpressed after viral integration (Cubie, 2007). It is located at the 3' end of the early region, downstream from the E2 gene. It is likely expressed through a polycistronic mRNA that also encodes E2.

E5 proteins, being small and hydrophobic, interact with transmembrane domains of receptor kinases, thereby altering their half-life and responses to ligands (IARC, 1995). In fact, the E5 protein has a transforming function because it alters signaling from growth factor receptors (Cubie, 2007). E5 proteins bind exclusively to a 16 kDa cellular protein, a component of protein ATPases (IARC, 1995).

E6 and E7 genes and proteins: E6 and E7 genes are located at the 5' of the early region and are expressed in the form of a poylcistronic mRNA transcribed from the E6/E7 promoter. The 2 reading frames are separated by a short variable distance. E6 genes of HPV types involved in malignancy are able to be spliced differentially to shorter proteins (IARC, 1995). The E6 gene co-operates with the E7 gene to stimulate cells into the S (Synthesis) phase of the cell cycle. Together with E7, it also slows down cell differentiation and increases the efficiency of transformation (Cubie, 2007).

E6 proteins of genital HPV's associate with and lead to the elimination of the tumour suppressor protein p53. E6 proteins can also repress and activate transcription of promoters through interactions with p53 or with the basic transcription initiation complex (IARC, 1995).

The E7 gene is the major transforming gene. It is capable of transforming cells independently of E6 (Cubie, 2007). The E7 gene of HPV-16 inhibits the binding of the retinoblastoma tumour suppressor protein (pRB) to E2 F transcription factors. It does this by forming a complex with pRB itself (IARC, 1995).

L1 and L2 genes and proteins: L1 and L2 proteins are part of papillomaviruses capsids. The later genes L1 and L2 are transcribed in BPV-1 and HPV-8 from a promoter in the LCR and, during the generation of mRNA's, sequences corresponding to the early genes have to be spliced out from the primary transcript. An equivalent promoter has not yet been reported for genital HPV's. Nuclear localization signals direct the transport of the capsid proteins into the nucleus where the viral particles are assembled (IARC, 1995).

Regulation of gene expression: This complex regulation is controlled by different cellular and viral transcription factors, different promoter usage, different splicing and different transcriptional termination and mRNA stability. The mechanisms have to achieve the following phenomena, many of which are deregulated during malignant progression of papillomaviruses lesions:

- Epithelial-specific transcription.
- Differential expression of papillomavirus genes during the differentiation of squamous epithelia, in particular the switch from early to late genes.
- Feedback control by papillomaviruses gene products, which may play an important role in the persistence of papillomaviruses infection.
- Response to physiological factors of the infected host on papillomavirus gene expression (IARC, 1995).

SCREENING AND DIAGNOSTIC TECHNIQUES FOR CERVICAL CANCER

Cervical cancer has been the first malignancy worldwide for which an effective method of screening has been introduced. A screening program is needed to make the public and the profession more aware of cervical cancer as the potential cause of even negligible gynaecologic symptoms (DiSaia and Creasman, 2002). A 50% of cases with invasive cervical cancer arise in women who are not effectively screened. Early diagnosis is also required because definite cure is readily attained when cervical cancer is minimal but almost impossible if the tumour has been given enough time to grow and metastasise (DiSaia and Creasman, 2002).

Cervical cytology

Overview: Cervical cytology (the Papanicolaou smear) involves collection of exfoliated cells from the cervix and microscopic examination of these cells after staining. This can be used not only to identify women with invasive cervical cancer but more importantly to identify precancerous lesions of the cervix. For this reason, cytology-based screening programs are the mainstay for cervical cancer prevention. In fact, cervical cytology is considered as a screening rather than a diagnostic test (IARC, 2005). A Pap smear has been shown to appreciably increase levels of inflammatory cytokines interleukin-12 (IL-12), interleukin-10 (IL-10) and tumour necrosis factor-" (TNF-") in the cervix. It is still unknown, however, whether these local Pap smear-associated inflammatory responses are one of the factors commencing long-term protection from HPV infection (Passmore *et al*., 2007).

There remains a question as regards the frequency at which a Pap smear must be performed. While, Pap smear screening in the United States (U.S.) was initially implemented as an annual test, analysis of data from large screening programs suggests that annual screening confers at best a minimal advantage over triennial screening. In light of this evidence, most U.S. professional medical societies have long since accepted that average women need not undergo Pap smear screening annually. At present, almost all industrialized nations have screening programs in which women are screened every 2-5 years. The majority of American women report being screened for cervical cancer more frequently than recommended (Sirovich and Welch, 2004). Yet, in general, women are not open to the idea of reducing the frequency of Papanicolaou (Pap) smears, however, because of their perception that annual screening is successful in reducing cervical cancer mortality (Smith *et al*., 2003).

Unsatisfactory, smears may be due to the inability to visualize the squamo-columnar junction as a result of cervical stenosis-a complication in women following treatment for Cervical Intraepithelial Neoplasia (CIN). This requires increased referral to colposcopy (Manivasagram *et al*., 2004).

Method: The ideal time for a cervical cytology examination is 2 weeks after the first day of the last menstrual period. Patients should avoid sexual intercourse and douching for 24-48 h before the examination. Also, women should not use any intravaginal products or medicine for several days before the smear is taken. Circumstances that may interfere with the interpretation of a cervical cytology test are active menstruation, significant cervical or vulvovaginal infections and a timing >8 weeks post-partum.

The cervix is first carefully inspected for grossly visible masses or ulcerations that may indicate an invasive cervical cancer. An adequate cervical cell specimen is then collected with the women in the dorsolithotomy position. A sterilized or single-use bivalve speculum of appropriate size is inserted into the vagina in such a way as to allow complete visualization of the cervical os and as much of the transformation zone as possible. The cervix should not be contaminated with lubricant of water-soluble gel that may obscure the smear. Therefore, the smear must be obtained before any bimanual examination.

If a spatula and a conical cervical brush are used, the spatula is first placed firmly against the ectocervix with the long projection extending into the endocervical canal. The spatula is then rotated several times 360° around the portio and removed. The entire transformation zone must be sampled. Transfer is best performed by using the spatula to thinly spread the cells from both sides of the spatula onto the glass slide. The endocervical canal is then sampled using the conical cervical brush, which is inserted into the canal and rotated 90-180° once. Material from both sides of the spatula should be spread onto the slide. Cell fixation must be performed within a few seconds of specimen collection to prevent air-drying. It is done by immersing the slide in alcohol or spraying it with a specially formulated spray fixative (IARC, 2005).

Liquid-based cervical cytology: Liquid-Based Cytology (LBC) is a way if improving the classical cervical cytology test. The cells scraped from the cervix are not spread onto a glass slide but are transferred to a liquid preservative solution that is transported to the laboratory, where the slide is prepared. A number of different LBC techniques are in use worldwide but the ThinPrep® and SurePath™ are the most widely used.

As an example, the ThinPrep method will be considered. Here, clumps of cells and mucus are broken

up by mechanical agitation and then, the liquid preservative solution is filtered through a membrane filter with a pore size specifically designed to trap epithelial cells whilst allowing red blood cells and inflammatory cells to pass through. The epithelial cells collected on the membrane filter are then transferred onto a glass slide and stained. This produces a thin, monolayer type preparation (IARC, 2005).

This method has several advantages over conventional cytology. Not only is there a more representative transfer of cells from the collection device to the glass slide (Watts, 2007), but there is also a decrease in the number of unsatisfactory cytology specimens. Moreover, residual cellular material can be used for the preparation of other glass slides or for molecular testing (IARC, 2005). In fact, liquid-based cytology specimens can be used for the immunohistochemical staining of p16, a biomarker for the primary screening of cervical cytology (Yoshida *et al*., 2004).

However, liquid-based cervical cytology is neither more sensitive nor more specific for detection of high-grade Cervical Intraepithelial Neoplasia (CIN 2 or 3) compared with the conventional Pap test (Arbyn *et al*., 2008). Suitable training for the collection of samples in a liquid solution could improve the adequacy of the sample and thus, the precision of the cytological diagnosis (Liverani *et al*., 2006).

Visual inspection: Visual Inspection (VI), also called down-staging or unaided visual inspection, consists of a clinical examination of the cervix using only a speculum and a light source. Visual inspection is subjective and therefore definition of test results should be standardized. In fact, test positivity is defined by the presence or the absence of specific characteristics, usually with low and high thresholds for positivity. Results are available without delay.

Over the last 10 years, the use of dilute (3-5%) acetic acid applied to the cervix before inspection, so known as Visual Inspection with Acetic acid (VIA) has been investigated. VIA involves naked eye inspection of the cervix one minute after application of a 3-5% solution of acetic acid using a cotton swab or a spray. Test positivity is based on the appearance of acetowhite areas in the transformation zone, close to the squamocolumnar junction or the os. The cervix is examinated using a bright light source such as a torch or a halogen focus lamp. VIA is also known as Direct Visual Inspection (DVI), Acetic Acid Test (AAT) and cervicoscopy.

Cervigography can then be performed. Cervicograms are replicate photographs of the cervix taken after application of 5% acetic acid, using a camera and internal light source. The images are projected onto a screen at a fixed distance to simulate magnification and are interpreted by a trained evaluator.

Dilute acetic acid causes a reversible coagulation of intracellular proteins, resulting in noticeable opacity and a decrease in the usual reddish hue imparted by the subepithelial vasculature. This effect, called acetowhiteninning, is not specific to cervical neoplasia and may also occur in immature squamous metaplasia and in inflamed, regenerating cervical epithelium. The degree of opacity produced varies according to the thickness of the neoplastic change in the epithelium and thus, according to the grade of intraepithelial neoplasia.

More recently, the application of Lugol's solution has been used and this technique is known as Visual Inspection with Lugol's Iodine (VILI). Lugol's iodine stains glycogen stored in cervical epithelial cells. Mature squamous epithelium stores more glycogen than either columnar epithelium or immature squamous columnar epithelium. The application of iodine to the cervix thus, results in black or dark brown staining or immature squamous epithelium. Columnar epithelium does not stain and retains its reddish hue. Areas of immature metaplasia stain a very light brownish hue, if at all. Neoplastic squamous epithelium contains little or no glycogen and does not stain with Lugol's iodine, taking a brown mustard or saffron yellow colour. Atrophic epithelium stains partially with Lugol's iodine, making interpretation difficult in post-menopausal women. A condylomatous lesion may noy stain or only partially stain with the solution. Areas of leukoplakia (hyperkeratosis) and areas partially denuded of squamous epithelium do not stain with iodine and remain colorless (IARC, 2005).

Colposcopy: Colposcopy is a procedure that allows illuminated stereoscopic and magnified (× 6-40) viewing of the cervix and vagina. The women is placed in the lithotomy position, with the cervix exposed with a bivalve speculum in place and various solutions-normal saline, 3-5% dilute acetic acid and Lugol's iodine-are applied in sequence. The aim is to examine the transformation zone.

The modern colposcope is a binocular microscope with a variable-intensity light source, providing a stereoscopic view of the cervix with a field of view and depth of focus that varies inversely with the magnification selected.

Terminology to describe the morphological findings in a standard way has evolved over the years; many descriptions have been classified as to the degree of abnormality and have been combined into a scoring system. Biopsies are obtained under colposcopic

visualization from the locations with the most severe changes, in order to histologically confirm the degree of severity of the neoplastic process (IARC, 2005).

HPV DNA testing

Overview: Research on the use of HPV DNA assays as a potential cervical cancer screening tool began in the late 1980s. There is much evidence that screening of women with both cytology and HPV DNA tests increases sensitivity for detection of Cervical Intraepithelial Neoplasia (CIN) 3 or cancer sufficiently to permit longer screening intervals than with cytology alone. This is one of the facts that led the US FDA to approve the second-generation hybrid capture assay (HC2) as an adjunct to cytological testing for women aged 30 years and older (IARC, 2005). Performance of HPV DNA detection methods as related to international standards will facilitate comparisons of data from multiple studies. Thus, the availability of international HPV DNA standards will contribute to the field of HPV prevention, diagnosis and treatment (Qiunt *et al*., 2006).

Techniques to detect the presence of HPV in cervical cell specimens have evolved considerable in the last 25 years from:

- Simple scoring of koilocytes in cervical smears to.
- Immunocytochemical staining to.
- Nonamplified nucleic acid hybridization methods such as dot blot, Southern blot and filter *in-situ* hybridization and finally to.
- Signal-amplified, immunoassay-based nucleic acid hybridization techniques such as the Hybrid Culture™ (HC) assay and a variety of Polymerase Chain Reaction (PCR) techniques (IARC, 2005).

The major obstacles to widespread acceptance of HPV testing in cervical cancer are its high unit cost and the fact that the technology is not in the public domain, as it is for cervical cytology. Various studies have been conducted as regards the cost-effectiveness of HPV DNA testing. In a study carried out by Kim *et al*. (2005) it was concluded that the use of HPV DNA testing, either for triage of women of any age with equivocal cytology results, or for primary screening in conjunction with cytology in women >30 years of age, has the potential to improve health benefits at a reasonable cost compared with current screening programs in the United Kingdom, The Netherlands, Italy and France (Kim *et al*., 2005). The most likely strategy to be cost-effective uses human papillomavirus testing to triage all women with an initial borderline and mild smear result and with a normal colposcopic finding (Legood *et al*., 2006), using cytology to follow-up women only with a negative test result for human papillomavirus (Bhaumik *et al*., 2004). The addition of testing for human papillomavirus in women with low grade cytological abnormalities resulted in a reduction in the rate of repeat smears, but an increase in rates of referral to colposcopy (Moss *et al*., 2006).

HPV DNA testing by home obtained samples is useful as a screening tool for cervical cancer (Nobbenhuis *et al*., 2002). In fact, self-assessment for HPV DNA is an easy, feasible and well-accepted method of testing for HPV and for cervical cancer in tertiary referral internal medicine outpatient clinics (Dannecker *et al*., 2004). However, participation in the screening programme remains the best option (Nobbenhuis *et al*., 2002).

Hybrid capture™ assay: Most clinical investigations of HPV testing have used first-or second-generation Hybrid Capture™ (HC) systems, the only HPV test currently approved by the US FDA. The HC system is a nucleic acid hybridization assay with signal amplification for the qualitative detection of DNA of high-risk HPV types in cervical specimens. It cannot however determine the specific HPV type present, since detection is performed with a combined probe mix.

The first-generation assay (HC1) was a tube-based detection system and probed for only nine of the high-risk HPV types. The second-generation HC system (HC2) has improved reagents and is based on a microplate assay lay-out that targets 13 high-risk HPV types. It uses long synthetic RNA probes that are complimentary to the DNA sequence of 13 high-risk HPV types. The initial reaction step denatures the exfoliated cells in STM, thus releasing host and any existing HPV DNA molecules to the solution. HPV DNA molecules then hybridize with the respective RNA probe, resulting in the formation of DNA-RNA hybrids reflecting the composition of HPV types present in the mixture. This hybridization step occurs inside the wells of a specially treated 96-well plastic microtitration plate previously coated with polyclonal IgG antibodies that are specific for RNA-DNA hybrids, regardless of sequence homology. Any such hybrids will then be captured by the solid-phase-bound antibodies.

After washing steps to remove unbound molecules, a solution of a conjugate reagent consisting of the same anti-RNA-DNA hybrid antibody covalently linked with the enzyme alkaline phosphatase is added to the wells. Conjugate antibody molecules will then bind to any solid-phase-bound hybrids. After further washing to remove unbound molecules, a solution containing a chemiluminescent dioxetane substrate is added to the wells. Cleavage of the substrate by the alkaline

phosphatase releases a luminescent reactiotion produced into the solution. The intensity of the light emitted is proportional to the amount of HPV DNA originally present in the specimen and is measured by a luminometer provided with the system. The reaction signal of each specimen is expressed on a scale relative to the average reactivity measured in triplicate wells with a positive control containing 1.0 pg of HPV 16 DNA per mL. Specimens yielding RLUs greater than or equal to 1.0 are considered positive.

The polymerase chain reaction: The Polymerase Chain Reaction (PCR) is based on the repetitive replication of a target sequence of DNA flanked at each end by a pair of specific oligonucleotide primers, which initiate the polymerase-catalysed reaction. Because of the exponential increase in the amount of target DNA sequence after a few reaction cycles of denaturation, annealing and extension, PCR has very high levels of molecular sensitivity. PCR is based on target amplification with type-specific or consensus or general primers. The latter are able to amplify sequences from several different HPV types. The amplified DNA products can be revealed with ethidium bromide staining following agarose or polyacrylamide gel electrophoresis (IARC, 2005). A PCR-based genechip (Easychip HPV Blot) detects more samples with HPV infection than HC2 in those patients with normal and ASCUS cytology for a broader HPV type range, but it is comparable to HC2 as regards the identification of oncogenic HPV genotypes (Huang *et al*., 2006).

Recently, the commercially manufactured PCR-based Roche AMPLICOR (AMP), which recognizes a group of 13 HR HPV types simultaneously (van Ham *et al*., 2005); and LINEAR ARRAY (LA) HPV tests have become available for HPV detection. Both are relatively easy to use and provide a rapid and standardized method for detecting and genotyping HPV, respectively. They exhibit greater sensitivity and lower specificity than HC2 for detecting high risk HPV. Hence, both the AMP and the LA tests can be used in the detection of high-risk HPV and to predict treatment success or failure, re-infection and/or new infections. They can also be used as an epidemiological tool for prevalence studies (Stevens *et al*., 2007).

CONCLUSION

The incidence of cervical cancer has declined in the past few years, largely due to the widespread execution of screening programs. The classical Papanicolaou smear has improved by the implementation of techniques such as liquid-based cytology, which give more precise results. More recently has been the development of techniques to detect the presence of HPV DNA in cervical samples. In fact, the Food and Drug Administration (FDA) approved the second-generation hybrid capture assay (HC2) as an adjunct to cytological testing for women aged 30 years and older. This age group was chosen because most HPV infections in the younger population clear up on their own, that is, a woman may be positive for HPV at a younger age but after age thirty, she becomes HPV-negative.

The benefits of HPV DNA testing in an ASCUS patient can be summarized as follows: Detecting the presence of a high-risk virus means that there is a greater chance of developing dysplasia and therefore colposcopy might be recommended. If a high-risk virus is not detected, it means that ASCUS was caused by infection with another organism, by infection with a low-risk HPV virus of it might be simply a false-positive result. In these cases, another Pap smear can be done to confirm results.

Yet, although screening for precancerous disease of the cervix has significantly reduced the incidence of cervical cancer, cervical cancer still causes of a certain degree of morbidity and mortality in many countries. It is important that all women undergo regular screening tests, because, if not so, the incidence of cervical cancer in a particular population will not decrease. Educational initiatives targeted to the female population from a young age about the benefits of screening tests will surely lead to further decrease in cervical cancer incidence that has been observed over the last years.

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