We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Molecular Tools for Detection Human Papillomavirus

Angela Adamski da Silva Reis,
Daniela de Melo e Silva, Cláudio Carlos da Silva and
Aparecido Divino da Cruz

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55710>

---

1. Introduction

The Human Papillomavirus (HPV) has been shown to play a causative role in anal, head and neck, oral, oropharyngeal, penile, vaginal, vulvar and cervical cancers. The last one is the second most common cancer among women worldwide [1-3]. Some types of HPV have been established as the central cause of cervical carcinoma [4-7]

Acquisition of HPV is very common, particularly among sexually active young adults, and incidence of infection with oncogenic HPV types appears to be higher than the incidence of infection with non-oncogenic types [8]. Oncogenic HPV types 16 and 18 and history of other concurrent sexually transmitted diseases were found to be significantly associated with progression to cervical cancer [1-13].

More than 100 HPV types have been identified and about 40 types can infect the genital tract. Worldwide, HPV 16 is the most common high-risk type, present in 50%, followed by HPV 18, present in 14% of cervical cancers [9]. Same types of HPV were more frequent in malignant than in benign lesions, and infection with high-risk types of HPV is now considered the major risk factor for the development of cancer of the uterine cervix [1].

Thus, the HPV infection is necessary for the development of the cervical cancer. The development of this cancer is considered to be a multistep process, where HPV is necessary but in itself an insufficient cause. Disease can only develop when there is persistent HPV infection of the cervical epithelium [3,9].

Cervical cancer is considerate 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. The incidence is highest in developing countries, largely as a

result of lack of screening programs and poor access to medical care [1]. The prevalence of HPV and the distribution of its types probably plays an important role as well. On the other hand, the relationship between others cancer types and HPV-associated is just emerging [10].

The variability in HPV-attributable proportions for non cervical cancers, in part, arises from differences in HPV detection methods across studies as well as from true geographic differences in HPV-attributable proportions [11]. Despite this variability, 90%–93% of anal cancers, 12%–63% of oropharyngeal cancers, 36%–46.9% of penile cancers, 40%–64% of vaginal cancers, and 40%–51% of vulvar cancers are potentially attributable to HPV infection [11–15].

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 programmes for detection of women at risk of disease progression, and also for incident infections [9,16]. Molecular detection of HPV provides a different approach to screening and patient management. In this chapter was described the diagnosis of HPV infection to screening cervical cancer and molecular tools to detect HPV-DNA/RNA.

2. The diagnosis of HPV infection to screening cervical cancer

Carcinoma of the uterine cervix is the second most common cancer among women worldwide, with very high mortality rates in developing countries. It was observed more than 20 years ago that some types of HPV were more frequent in malignant than in benign lesions, and infection with high-risk types of HPV is now considered the major risk factor for the development of cancer of the uterine cervix [1]. Oncogenic HPV types 16 and 18 and history of other concurrent sexually transmitted diseases were found to be significantly associated with progression to cervical cancer [5].

Studies have demonstrated a strong association between lifetime number of sexual partners and genital HPV acquisition. The acquisition of new sexual partners continues throughout all age groups. In addition, studies have shown consistently that the risk of cervical cancer can be predicted as much by a woman's own sexual behaviour as by the sexual behaviour of her husband/partner. The presence of HPV DNA in the penis and urethra of her sexual partner(s) is directly related to her HPV carrier status and therefore her risk of developing cervical cancer [13–15].

Of the genital HPVs, which are sexually transmitted, 15 are categorized as high risk and are considered the causative agents of most cervical cancers, with over 99% of cervical lesions containing viral sequences [1]. The remaining viral types are rarely found in malignancies. High-risk HPVs (HR-HPV) are also associated with many vulvar, anal and penile carcinomas and contribute to oral cancers [17], 2006). Additionally, these cancers, in contrast to cervical cancer, appear to be preferentially associated with HPV16 [11,14]. For instance, in the subset of penile cancer attributed to HPV infection, HPV 16 was found in 60,23% of cases [18]. A vaccine has recently been introduced that can prevent the initial infection by two of these high-risk types, HPV 16 and 18, which are responsible for about 70% of cervical cancers [19].

The process by which HPV facilitates tumour initiation and fosters tumour progression is an exceptional model to understand the development of many other human cancers and also allows identification of additional signalling pathways targeted in malignant progression [19]. The association between HPV and human cancer was first proposed more than three decades ago by Harald zur Hausen, and he was honored with one of two 2008 Nobel Prizes in Medicine for his isolation and characterization of HPV 16 in 1983 and later HPV 18 in cervical cancer [20]. The award recognized not only the importance of his discovery in the eventual documentation of the etiology of HPV in cervical and a number of other cancers, but also the importance of the application of his discovery to the clinical use of HPV testing and in implementation of the HPV vaccine.

Additional, many studies have demonstrated the direct role of HPV infection in the development of several human cancers [12-15,21]. HPV 16 and HPV 18 are the most frequently found HPV types in cervical cancers worldwide, being detected in approximately 50 and 20% of the cases, respectively [2-22]. For this reason, the majority of the biological studies were focused on these two HPV types.

The viral genomes are replicated in synchrony with cellular DNA replication. After cell division, one daughter cell migrates away from the basal layer and undergoes differentiation. Differentiation of HPV-positive cells induces the productive phase of the viral life cycle, which requires cellular DNA synthesis machinery. The expression of E6 and E7 deregulates cell cycle control, pushing differentiating cells into S phase, allowing viral genome amplification in cells that normally would have exited the cell cycle. The late-phase L1 and L2 proteins encapsidate newly synthesized viral genomes and virions are shed from the uppermost layers of the epithelium [19].

The induction of hyperproliferation by the E7 oncoprotein triggers apoptosis, which is blocked by the actions of the E6 oncoprotein. The cooperative actions of E6 and E7 efficiently immortalize cells and this process is augmented by the actions of the E5 protein. The ability of E6 and E7 to target crucial regulators of proliferation, apoptosis, immortalization and genomic stability collectively promotes the emergence of a clonal population of cells with a growth advantage and an increased propensity for transformation and malignant progression [19].

The best-characterized HPV 16 E6 activity is its ability to induce degradation of the tumor suppressor protein p53 via the ubiquitin pathway. This cellular protein is a transcription factor that can trigger cell cycle arrest or apoptosis in response to a large variety of cellular stresses, such as hypoxia or DNA damages. Overall, the role of p53 is to ensure the integrity of the cellular genome, preventing cell division after DNA damage or delaying it until the damage has been repaired. The induction of p53 degradation appears to be an exclusive feature of E6 proteins from the HR-HPV types [23].

Codon 72 polymorphism on the 4th exon of TP53 is involved in multiple steps of carcinogenesis and may also account for genetic differences in susceptibility to cancer [24-26]. This most common polymorphism results in a non-conservative change at codon 72 of an arginine to a proline within a proline - rich region of p53 which is known to be important for the growth suppression and apoptotic functions [25]. It has been demonstrated that the TP53 polymor-

phism varies according to ethnic and geographical distribution, like most human genetic polymorphisms [24].

A meta-analysis of such studies revealed that the arginine homozygous genotype is associated with an increased risk of invasive cervical cancers, but not with squamous intraepithelial lesions supporting the hypothesis that the p53 codon 72 polymorphism may have a principal role in progression to HPV-related cancer, rather than in initiation of the neoplasia [27].

For prevention and early detection of cervical cancer, it is important to detect not only cervical intraepithelial changes, but also to identify the presence of HR-HPV and its type as well. If the results of HR-HPV test are positive, the possibility of cervical intraepithelial neoplasia (CIN) can be prognosticated even if there are no cytologic changes in the cervix. The possibility for regression of CIN 2 cervical changes caused by HPV type 16 is lower compared with cervical changes caused by other HR-HPV types. The risk of mild cervical changes leading to severe cervical changes (CIN 3) is higher when detecting HR-HPV types, especially HPV type 16, compared with lower-risk HPV types. HR-HPV-positive women, even without cytologic changes, have a 210-fold higher risk of developing CIN 3 in 6 years as compared with HR-HPV-negative women [28].

Starting in the late 1960s significant advances were made in understanding the cellular changes leading to invasive cervical cancer, but it was not until 1976 that Meisels and Fortin first established HPV as the etiologic agent in an abnormal cervical cytologic finding (koilocytotic atypia) [20]. The advent of screening to identify and treat cervical cancer precursor lesions, CIN, has led to a substantial reduction in the incidence of cervical cancer in those countries where routine screening is in place. Conversely, most cervical cancer-related mortality occurs in countries where there is no routine cervical screening, although Pap's smear is a cost effective screening method in low resource settings [10].

Cervical screening is considered to have been the most effective cancer-screening test ever introduced and in developed countries with organized programmes. A successful screening programme however is dependent on understanding and acceptance of the need for a particular test, the need for further investigation of abnormalities and particularly, the need for quality assurance of all parts of the system [29].

In the evaluation by IARC in 2005 was concluded that there is sufficient evidence that screening women ages 35 to 64 for cervical cancer precursors by conventional cytology every 3 to 5 years within high-quality programs reduces incidence of invasive cervical cancer by at least 80% among those screened [6]. Despite the large amount of data available on the value of HPV-DNA testing for the detection of cervical cancer precursors, both in the primary cervical screening and in the management of 'borderline' or Atypical Squamous Cells of Undetermined Significance (ASCUS) cytology, HPV-DNA tests have not always and correctly been translated into clinical practice by clinicians and within national cervical screening programs [30-31].

Although Pap's smears have reduced the incidence of cervical cancer by over 80% in the United States, cervical cancer is the second leading cause of cancer deaths in women worldwide, and effective implementation of the HPV vaccine and continued screening should dramatically reduce the incidence of these cancers [19]. Cervical cytologic testing or colposcopy is an

acceptable method for managing women over the age of 20 years with ASCUS, but HPV-DNA testing is the preferred approach. Several population-based studies have established that tests for HR-HPV DNA have higher clinical sensitivity than cytology in detecting cervical intraepithelial neoplasia (CIN) of grades 2 and above (CIN2+), and that combined HPV and cytology testing shows the highest negative predictive values (NPV) for CIN2+ [31-69]. Thus, the HPV DNA test should be used in conjunction with Pap's smear test wherever feasible and affordable or potentially as a stand-alone test [32], because both combined has been shown to greatly improve the ability to detect pre-cancerous states [33].

In general, the prevalence of HPV is higher in young women compared to women over 30 years [34-35]. Most HPV infections are transient [9,36] and natural history studies have shown that HPV DNA is detectable in cells from the cervix for less than a year in most infected women. Therefore, the presence or absence of HPV DNA at a single time point is a poor indicator of lifetime exposure. To mitigate this problem, serological tools to detect HPV antibodies have been developed.

However, the serological assays have only limited accuracy and HPV cannot be grown in conventional cell cultures. As infection with HPV is followed by a humoral immune response against the major capsid protein [37], 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 [9,38].

The Hybrid Capture Assay I (HC1) was first introduced by Digene in 1995 [39]. HC1 was a relatively fast, liquid hybridization assay designed to detect 14 HPV types divided into high-risk types (HPV 16, 18, 31, 33, 35, 45, 51, 52 and 56) and low-risk types (HPV 6, 11, 42, 43, and 44). Initially, this was to be used to augment the sensitivity of conventional Pap testing and to provide a meaningful negative predictive value for assessing cervical dysplasia [20].

The second generation of Hybrid Capture Assay, the hybrid capture-II (HC2) DNA test - Digene (Now Qiagen, Valencia, Calif Gaithersburg, MD, USA), which uses a micro titer plate instead of tubes and has been approved by the US Food and Drug Administration (FDA) for DNA- HPV identification [40], as an adjunct to cervical screening in the US women in aged 30 years and over. HC2 is a semiquantitative measure of viral load relative to 1pg/ml and uses RNA-labeled probes for targeting DNA sequences from 13 high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) 5 low-risk types (6, 11, 42, 43 and 44) [7,40-41].

Although its use has become the standard in many countries, it has several limitations including the inability to identify specific types and the lack of internal control for the amount of input DNA [7,40-42]. In addition, the reliance on sample volume as a proxy for cellularity may give rise to false negatives in samples with few infected cells. The lack of a negative control within the test also prevents detection of false negatives due to procedural or reagent problems. Another source of concern is the fact that the HR-HPV probe set is not all-inclusive. Women with unusual types may have a true-negative HC2 test yet still harbor a virus capable of causing cancer [43].

Studies comparing HC2 and Polymerase Chain Reaction (PCR) results have also shown that the hybrid capture probes are not entirely type specific [43]. The detection limit of approxi-

mately 5000 genome is the power of HC2 and it makes it less sensitive than PCR and cross-reactivity of the two probe cocktails can reduce the clinical relevance of a positive result [20,40].

Despite the HC2 present some limitations [33], this assay has shown high sensitivity for the detection of CIN of grade 2 and worse (CIN2+) and it was recently recommended to be used as a benchmark for performance assessment of new candidate HPV tests for primary cervical cancer screening in women of 30 years and older. The emergence of competing platforms like the Linear Array (Roche) and INNO-LiPA HPV (Innogenetics) has led to the development of some new elements by Qiagen that are designed to counteract perceived advantages of alternative tests [43]. These other platforms are commonly used in the HPV typing assays and follow up of persistent infections to monitor the presence of specific HPV genotypes [44].

Has been demonstrated that HC2 has proven its effectiveness in large clinical trials and everyday practice, but the search continues for markers with superior specificity for high-grade disease without excessive corresponding loss of sensitivity. A number of new assays have been developed for molecular and immunostaining platforms with the intention of meeting this need [43].

As a screening test, cervical cytology for CIN has reduced the incidence of mortality worldwide [45], however this methodology has a limited sensitivity. So, as described by [46] a shift from conventional cytology to a molecular approach integrated into cervical cancer screening is the most likely solution to the goals of improved screening in both the developed and developing world. Therefore, molecular tests have become available for clinical and research purposes in response to the need for identifying infection during earlier stages and improving patient follow-up [9,40].

A modified, experimental Hybrid Capture assay named Hybrid Capture 3 uses RNA probes, as in Hybrid Capture 2, but in combination with biotinylated capture oligonucleotides that are directed to unique sequence regions within the desired target to increase test specificity [47]. The assay has been developed further to reduce cross-reactivity while maintaining sensitivity and for use either on DNA or RNA as targets. A recent comparison study concluded that, at the optimal cut-off points, Hybrid Capture 2 and 3 had similar screening performance characteristics for high-grade lesions diagnosed at the enrolment visit [48].

Hybrid Capture 3 (HC3) is being evaluated as the next generation of hybrid capture clinical assays that target 13 oncogenic HPV types for the detection of cervical precancerous cervical intraepithelial neoplasia grade 3 (CIN3). A primary technical distinction between HC3 and HC2 is that HC3 employs a biotinylated DNA oligonucleotide specific for selected HPV DNA sequences for the capture of the DNA-RNA complexes on streptavidin-coated wells, whereas HC2 uses wells coated with polyclonal antibody against DNA-RNA complexes for hybrid capture. The use of capture oligonucleotide instead of an immobilized antibody also diminishes the possibility of nonspecific RNA-DNA hybrids, present as the result of improperly alkali-denatured specimens, from binding to the microplate well and consequently may reduce false positivity for HC3 compared to HC2 [48].

This ratio assay may reflect the natural pattern of HPV mRNA expression that occurs during the progression of disease in cervical epithelium. After initial infection, polycistronic, pre-mRNA

is transcribed from HPV DNA that exists either as circular, extrachromosomal episomes or as DNA that is integrated into the chromosome. As pre-malignant lesions progress, the abundance of mRNAs that encode the oncogenes, such as E6 and E7 (E6–7), may increase and the mRNAs encoding non-oncogenic HPV proteins, such as E2 and L1, may decrease [49]. The incidence of HPV integration may reduce further transcripts encoding E2 and other downstream genes, such as L1, because integration usually occurs at the E2 loci [50]. The E2 gene product is an important down-regulator of oncogenic E6–7 expression [19]. Thus, lower E2 levels may correlate with disease progression. Therefore, it may be useful to measure the ratio of E6–7 over E2 transcripts in cervical specimens and compare this ratio with the severity of disease [51].

In the study [48] was compared the performance of a prototype version of the Hybrid Capture 3 (HC3) human papillomavirus (HPV) DNA assay to the current generation Hybrid Capture 2 (HC2) assay, both of which target 13 oncogenic HPV types, for the detection of cervical intraepithelial neoplasia grade 3 and cancer (CIN3+) with cervicovaginal lavage specimens collected at enrollment into a 10-year cohort study at Kaiser Permanente. The authors suggest that HC3 may be a slightly more sensitive, equally specific test for the detection of CIN3+ over the duration of typical screening intervals compared to its predecessor, HC2. The increased sensitivity of HC3 compared to HC2 appears to be the result of increased detection of CIN3+ in women who were 30 years of age or older and were cytologically negative. They emphasize that further validation studies of HC3 are needed with more clinically relevant cervical specimens.

Diagnosis of HPV infection relies on the detection of the viral DNA in clinical samples; thus accurate detection and genotyping of HPV are of critical importance for determining the prevalence of HPVs in a given population and for determining the risks associated with infections of a particular type [52]. The first evaluation of the use of HPV testing in a potential clinical application was published in 1989 by Tidy et al. on the detection of HPV 16 by PCR both in normal and in dyskaryotic smears from 21 women. The results was sufficiently compelling to predict that HPV testing might eventually supplement cytologic analysis of cervical samples in screening [20].

PCR is a highly sensitive technique and allows testing on samples with less tissue or fewer cells [7,46]. Multiplex HPV genotyping methods based on hybridization to fluorescently labeled beads have also been reported [53]. These methods are capable of detecting very small copy numbers of virus, and extremely high sensitivities have been reported. However, because HC2 has been established as the criterion standard for clinical treatment algorithms, such high positive rates are actually a drawback rather than an advantage for clinical use. The relative complexity of PCR-based methods, concerns about contamination of the laboratory by PCR products, and patent protection on some aspects of the technology have further hindered widespread adoption of this approach [43].

The sensitivity and specificity of PCR based methods vary, depending mainly on the primer set, the size of the PCR product, the reaction conditions and efficacy of the DNA polymerase used in the reaction, the spectrum of HPV types amplified, the ability to detect multiple types and the availability of a type-specific assay. PCR can theoretically produce 109 copies from a single double stranded DNA molecule after 30 cycles of amplification. Therefore, care must be taken to avoid false-positive results derived from cross-contaminated specimens or

reagents. Several procedures are available to avoid the potential problems of using PCR protocols for HPV DNA detection [30].

The limitations associated with PCR-based HPV-DNA detection are related to primer selection and optimal protocol standardization [46,54]. The PCR assay most likely to be amendable to broad screening and/or surveillance applications are based on consensus primer amplification of a broad spectrum of genotypes which are subsequently differentiated by type-specific oligonucleotide probe hybridization. The most commonly used consensus PCR target is the highly conserved L1 open reading frame (ORF), of genital HPV genomes [52]. Among these are the single pair of consensus primers GP5/6 [55] and its extended version GP5+/6+ [35,56] and the MY09/11 degenerate primers [57] and its modified version, PGMY09/11 [30,58] and SPF 10 [59]. In addition, none of these assays can be automated or deployed on a high-throughput platform, features that are essential for an assay intended for use with a large volume of patients [60].

The viral nucleic acid must be preserved to avoid false-negative results caused by degradation by endogenous endonucleases. This is especially important when analyzing HPV-RNA transcripts. To assess the integrity of genomic DNA in the specimen and its suitability for molecular analysis, adequate controls, such as β -globin gene amplification or spiking of the sample with known positive material, are crucial. Several commercially available sampling kits, originally intended for cytology (e.g. PreservCyt, Cytyc Corp.) adequately preserve nucleic acids for molecular diagnosis even after prolonged storage at ambient temperatures [61].

The commercial HPV assays are based on L1 or E1 PCR for high-risk HPV DNA detection and genotyping are now available from different companies: AmplicorR and Linear Array (Roche Molecular Systems, CA, USA), INNO-LiPAR (Innogenetics, Ghent, Belgium), PapiloCheck (Greiner Bio-One GmbH, Germany), Multiplex HPV Genotyping Kit (Multimetrix GmbH, Heidelberg, Germany) [Table 01]. After amplifying parts of the L1 region, such assays hybridize the resulting PCR product to a detection and visualization unit (microarray or a reverse line blot) [62].

HPV-DNA assays can be performed using the same specimen as used for cytological examination, which is an important logistic aspect of routine clinical testing. However, a cervical scrape is only a small sample of the cervical epithelium and sampling errors may influence cytology grading. Only a portion of the cervical cell suspension is used for DNA isolation with only a fraction of the isolated DNA being used for specific DNA detection. Therefore, if a specimen only contains a limited number of HPV-DNA copies, sampling errors may produce inconsistencies even in a sensitive assay. Furthermore, the outcome of a HPV-DNA assay can vary depending on the menstrual cycle [9].

Luminex (xMAP) suspension array technology is based on polystyrene beads that are internally dyed with various ratios of two spectrally distinct fluorophores. Different molecules such as individual oligonucleotide probes can be coupled to different bead sets with specific absorption spectra. These sets are combined to a suspension array and allow up to 100 different probes to be measured simultaneously in a single reaction (multiplexing) [62].

This technology can potentially be fully automated, dramatically decreasing the personal cost component of the assay; this assay has been used for the genotyping of 45 HPV types with

PGMY09/11 PCR, 22 HPV types with GP5+/6+ PCR 15 HPV types with YBT L1/GP-1 PCR, and 18 HPV types with GP5+/6+ PCR. These multiplex HPV genotyping (MPG) methods have been compared with other well established HPV detection methods such as HC2, restriction fragment length polymorphism (RFLP) and DNA chip technology for the evaluation of their performance. However, none of these well established assays are perfect and suitable for the “gold standard”. Sequencing gives the most conclusive genotype information, although it is the most labor intensive. However, sequencing is the most desirable way to validate HPV genotyping methods [62].

Assay name	HPV genotypes detected	Detection technology	DNA and HPV gene detected	Producer
Hybrid capture 2 (HC2)	High-risk 16,18,31,33,35,39,45,51,52,56,58,59,68 Low –risk 6, 11, 42, 43, 44	Sandwich capture molecular	DNA, L1	Qiagen (MD, USA)
Cervista	16, 18 and bulk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	Invader chemistry	DNA, L1, E6, E7	Hologic (WI, USA)
Cobas HPV test	16, 18 and bulk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	Real-time PCR	DNA, L1	Roche (Rotkreuz, Switzerland)
Abbott´s Real Time HPV	16, 18 and bulk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	Real-time PCR	DNA, L1	Abbott (IL, USA)
Clinical Array	6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, 89.	PCR/microarray	DNA, L1	Genomica (Madrid, Spain)
Linear Array	6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 71, 71, 72, 73, 81, 82, 83, 84.	PCR/reverse hybridization line blot	DNA, L1	Innogenetics (Gent, Belgium)
INNO-LiPA Genotyping Extra	6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 69, 70, 71, 73, 74, 82.	PCR/reverse hybridization line blot	DNA, L1	Innogenetics (Gent, Belgium)
PapilloCheck	6,11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44/55, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82	PCR/microarray	DNA, L1	Greiner BioOne (Frickenhausen, Germany)

* Modified from [62]

Table 1. Commercial HPV-DNA assays

3. HPV nucleic acid detection

Detection of HPV E6/E7 messenger RNA (mRNA) is an indicator of HPV oncogenic activity and may be used as a clinically predictive marker to identify women at risk of developing high-grade cervical dysplastic lesions and cervical carcinoma [63]. This method has been proposed as a more specific marker for cervical dysplasia and cancer than HPV DNA [64]. Several assays have been designed to detect mRNA of the E6 and E7 transforming genes of HPV. The level of E6 and E7 gene expression is increased in high-grade lesions compared with low-grade lesions. These tests have high specificity for detecting disease and could potentially serve as functional discriminators between high-risk and low-risk infections [19].

Initial studies comparing HPV DNA and RNA detection (using consensus PCR and PreTect HPV-Proofer) in women with ASCUS and mild dysplasia on cervical cytology showed that twice as many women were positive for HPV DNA compared with those with HPV RNA positivity. They also demonstrated that while both tests were highly sensitive for detection of CIN2+ (85.7%), HPV RNA detection was far more specific for detection of high-grade lesions (84.9% vs 50%) [65].

The PreTect HPV-Proofer (NorChip AS, Klokkestua, Norway) is a commercially available real-time multiplex nucleic acid sequence-based amplification (NASBA) assay, also called NucliSENS EasyQ (bioMérieux, Marcy l'Etoile, France). This assay is based HPV detection using HPV DNA plasmids and detects E6/E7 mRNA of the five most common HPV types, HPV16, 18, 31, 33, and 45 [64,66]. These five types have been shown to account for about 82% of cervical cancer worldwide, but their prevalence varies between different geographical regions [67].

In comparison to HPV DNA tests, NASBA-based HPV detection showed better results in terms of specificity for high-grade cervical lesions, while its sensitivity is lower. Currently, it is difficult to know whether the differences in diagnostic accuracy are a result of the larger type detection range and high analytical sensitivity of the HPV DNA tests. To elucidate this issue, experimental confirmation that RNA is the sole target of HPV NASBA is required [64].

NASBA-positive women are 69.8 times more likely to be diagnosed with CIN2+ within 2 years of testing than NASBA negative women. Thus, the addition of HPV mRNA triage to cervical screening programmes may decrease the incidence of false-negative results while also allowing the time interval between screening events to be increased for those women who are HPV DNA and RNA negative [63]. Several studies on the relative performance of NASBA have been conducted in Europe with an indication that this test is more specific than other tests, including the APTIMA and HC2 assays to identify CIN 2+ [65,68-70]. This indicates that the NASBA assay can be used reliably to obtain simultaneous type-specific information for the five genotypes targeted by the test. This is an appealing feature given the indication for the identification of types 16 and 18 in risk stratification and better clinical management of women with a positive HPV test [71].

On the other hand, there have been reports that the NASBA technique can detect DNA, causing false-positive results [64,72]. However, NASBA can serve as a better triage test than HPV DNA

to reduce colposcopy referral in both ASCUS and low grade squamous intraepithelial lesion (LSIL). It is also more efficient than cytology for the triage of HPV DNA-positive women. Nevertheless, its low sensitivity demands a strict follow-up of HPV DNA positive-mRNA negative cases [73].

APTIMA (Gen-Probe, San Diego, CA) is a commercially available mRNA test for HPV, which detects mRNA of HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), based on transcription-mediated amplification [66,74]. There is indication that the APTIMA test has clinical sensitivity similar to that of HC2 [74], but with higher specificity for detection of dysplasia and improved clinical specificity over that of HC2, which is designed to detect DNA of the same HPV types, except for type 66 [66]. The Germany study concluded that APTIMA had a sensitivity similar to but a specificity significantly higher ($P < 0.0001$) than the HC2 test for the detection of CIN2+. The AHPV assay was significantly more sensitive and significantly more specific than cytology for the detection of disease [75]. The assay is sensitive and very specific for detection of high-risk HPV, which may improve patient management and reduce the cost of care. However, APTIMA does not specify the individual HPV types detected [76].

In study carried out as part of a multicenter study in Canada which was assessed the clinical usefulness of testing for E6/E7 mRNA and other molecular biomarkers in cervical cancer screening in comparison with HPV DNA testing and cytology. A point worth noting is that 61 (15.9%) of 384 CIN 2+ cases were found in women with normal cytology at the time of enrollment, and 70.5% and 86.9% of these were positive by NASBA and HC2, respectively. This reinforces the importance of incorporating HPV testing or repeat cytology in cervical cancer screening. Also, a separate analysis of unsatisfactory cytology indicated an enriched population of CIN 2+ [71].

This reflects the inherent limitation of cytology-based evaluation. Regardless, the positivity with NASBA or HC2 was dependent on the number of genotypes covered by the respective tests and their prevalence in different grades of cytologic and histologic lesions. Furthermore, the lesion progression is more strongly associated with types 16 and 18, and there are indications that an HPV test that distinguishes types 16 and 18 from other oncogenic types may be more useful as it could identify women at greater risk of cervical cancer [71].

Microfluidic approaches in diagnostics achieve significant reagent volume reduction and thus cost-drive innovation, potentially achieving widespread penetration in non-hospital, non-specialized environments. However, microfluidic approaches are not without their challenges. The field of “lab-on-a-chip” (LOC) diagnostics has grown rapidly from this basic need, and it is fast accelerating towards a “sample-in answer-out” platform for molecular diagnostics. NASBA technology with real-time fluorescence measurement was adapted to detect HPV mRNA in cervical specimens and cervical cell lines. The isothermal nature of NASBA greatly simplifies amplification strategies for nucleic acid detection on chip. This platform has huge potential within “point-of-care” (POC) diagnostics as up to 16 different targets can be detected simultaneously for each clinical sample analyzed [77].

In recent study from Norway, the prototype NASBA platform presented amplification efficiency of has been compared to an industry gold standard for HPV detection with encouraging

results. By this, we have demonstrated the subcomponents of a complete integrated in vitro diagnostics system: from clinical sample input to sample preparation to amplification to detection, thus advancing towards a “sample-in, answer-out” diagnostic platform. The prototype NASBA platform combined extraction and amplification in a microfluidic device consisting of extraction and NASBA chips. In addition, the adopted NASBA method offers the unique characteristics of isothermal amplification, greatly simplifying the thermocycling requirements for the system: effectively allowing a “blackbox” technology to be developed encompassing amplification and detection simultaneously in a real-time format. The Authors described this technology platform is not limited to diagnostics of cervical precancer and cancer, but it has enormous potential in the monitoring and diagnosis of gene activity in areas such as infectious disease, oncology, immune response to allergens, immunotherapies, and chemotherapies [77].

The mRNA assays with real-time PCR may be a useful tool in investigation of as well as in primary screening for cervical neoplasias, and it might be worthwhile to consider which genotypes to include in further investigations to optimize sensitivity and specificity, especially in a post vaccine era, when it may be necessary to reconsider HPV testing strategies [66].

3.1. MicroRNA analysis in Human Papillomavirus (HPV)

MicroRNAs (miRNAs) are noncoding regulatory RNAs 18-25 nucleotides in size that are derived from RNA polymerase II (pol II) transcripts of coding or noncoding genes. MiRNAs most often function by binding to the 3' UTRs of target messenger RNAs, whereby they induce mRNA degradation or translational repression [78]. Many miRNAs are tissue- or differentiation-specific, and their temporal or short-lived expression modulates gene expression at the posttranscriptional level by base-pairing with complementary nucleotide sequences of target mRNAs [79-80]. The functions of miRNAs are still largely unknown, but it seems that they are important in the regulation of cellular gene expression and behavior [81,82].

As of August 2012, the miRBase database (<http://www.mirbase.org/>) [112] had collected 21,264 entries representing hairpin precursor pre-miRNAs. Human genome contains ~ 416 miRNA genes encoding 1048 distinct mature miRNAs from every chromosome except Y. Approximately 113 miRNA genes encode a cluster of miRNAs and produce ~ 390 miRNA sequences. Bioinformatics prediction shows that each miRNA targets ~ 200 RNA transcripts directly or indirectly, and up to one-third of the total number of human mRNAs are targets of more than one miRNA [81]. So, the actions of miRNAs exert profound effects on gene expression at the posttranscriptional level in almost every biological process. However, miRNA expression itself, similar to any other transcription mediated by pol II, is regulated both at the transcriptional and posttranscriptional levels.

Many cellular transcription factors, including c-Myc, p53, and E2F, have been described to regulate miRNA transcription. Oncogenic HPV E6 induces degradation of p53 and E7 mediates degradation of pRB to release E2F from the pRB–E2F complex, it is conceivable that oncogenic HPV infection causes aberrant expression of cellular miRNAs [81]. Other factors involved in miRNA maturation and processing after transcription are Drosha (an RNase-II endonuclease that produces pre-miRNA from pri-miRNA), DGCR8 (DiGeorge syndrome critical region gene 8, a double-stranded RNA-binding protein needed for Drosha activity), exportin 5 (for pre-

miRNA export), Dicer (an RNase-III enzyme that produces mature miRNA from pre-miRNA), TRBP (a Dicer partner), and Ago2 (a major component for RISC) [81,83-84].

In the study [81] was profiled 455 miRNAs via miRNA microarray in 4 cervical cancer tissues and 4 normal cervical cancer tissues and they found significant overexpression of 18 miRNAs and underexpression of 15 miRNAs cervical cancer tissues compared to normal cervical cancer tissues [85]. In the analysis from 157 cellular miRNAs via the TaqMan MicroRNA Human Early Panel Kit (Applied Biosystems) in 10 early stage squamous cell carcinomas and 10 normal cervical samples. The authors found overexpression of 68 miRNAs and underexpression of two in the cervical carcinomas versus normal cervical samples [86].

A recent review published [81] presented that cervical cancer represents a unique tumor model for understanding how viral E6 and E7 oncoproteins deregulate the expression of the miR-15/16 cluster, miR-17-92 family, miR-21, miR-23b, miR-34a, and miR-10b/93/25 cluster via the E6-p53 and E7-pRb pathways. miRNAs may influence the expression of papillomavirus gene in a differentiation-dependent manner by targeting viral RNA transcripts. Genome-wide profiling of miRNA signatures has indicated that aberrant, increased or decreased, miRNA expression is common in most human tumors [83,86]. The table 2 [81] presented a summary of miRNA expression profiling studies in cervical cancer, as illustrated below, in three distinct studies.

In the study conducted in 2011 was found significant overexpression of 18 miRNAs and underexpression of 2 miRNAs in cervical cancer tissues compared to normal cervical tissues via TaqMan MicroRNA arrays. Those authors demonstrated, via individual qRT-PCR assays, significant overexpression of miR-16, miR-21, miR-106b, miR-135b, miR-141, miR-223, miR-301b and miR-449a, and underexpression of miR-218 and miR-433 in cervical cancer and dysplasia from normal cervical compared to normal cervical tissue. Their results showed that miR-21, miR-135b, miR-223 and miR-301b were overexpressed in the cervical cancer tissue compared to both normal and cervical dysplasia tissue. So, those authors concluded that such miRNAs are good candidates for markers of progression from normal to dysplasia to cancer [85].

Another recent study published in 2011 was analyzed six paired normal and cervical cancer tissues by using the same miRNA array platform and showed an increased expression of 12 miRNAs and decreased expression of 11 miRNAs, as presented in table 2 [87]. The study presented by [88] found increased expression of miR-21 in cervical cancer and a decreased expression of both miR-143 and miR-145. Those data together indicated that cervical cancer expresses no or very little of the miR-143/145 cluster. A PCR-based miRNA assay was used to analyze 102 cervical cancer samples [89] and the authors identified miR-200a and miR-9 as two molecular markers that could be used to predict cervical cancer survival.

In the analysis [90] was investigated 802 unknown and 122 predicted human miRNAs via CaptilBio mammalian miRNA arrays V 3.0 in 13 HPV-16 and HPV-18 positive cervical cancers and their adjacent normal tissues. The authors found that miR-141 is overexpressed and miR-218 is underexpressed in cervical cancer compared to normal cervical tissue. In the study [111] analyzed miRNA expression via microarray in 5 cervical squamous cell carcinomas that appeared to be HPV-negative and 5 normal cervical tissues and they found that miR-21 to be overexpressed in the cervical cancer samples.

MiR-21 is the most highly overexpressed miRNA in numerous cancers and it down regulates the tumor suppressor PTEN in non-small cell lung cancer and in hepatocellular cancer, in which PTEN regulation leads to overexpression of matrix metalloproteinases MMP2 and MMP9 [85]. These proteins promote cellular migration and invasion. MiR-21 also targets the tumor suppressor genes tropomyosin I (TPM1), programmed cell death 4 (PDCD4) and maspin, and the latter two have been implicated in tumor invasion and metastasis [91]. Increased miR-21 expression in cervical cancer may be attributable to both E6 and E7. Such viral oncoproteins play important roles in regulating cellular miRNA expression and their function can be reduced due to epigenetic modification of miRNA genes. Identification of a panel of miRNAs that can be used as early biomarkers in cervical cancer is potentially useful to determine disease behavior and prognosis. They may also provide new targets for anticancer therapy [85].

Until now, there are evidence indicating that HPV regulation of cellular miRNA expression most likely occurs through viral E6 and E7 [81], although oncogenic HPVs do not produce viral miRNAs, they are responsible for the aberrant expression of oncogenic or tumor suppressive miRNAs. High-risk E6 and E7 interact separately with several dozens or even hundreds of cellular factors and these interactions could lead to increased or decreased expression cellular miRNAs. Some of the altered miRNA expression could be a result from both E6 and E7. It is known that the main function of oncogenic HPV E6 is to target p53 for degradation [81-93]. As a transcription factor, p53 plays an important role in the transcription of numerous coding and noncoding genes [92-94] and it seems that oncogenic HPV is capable of regulating the expression of many cellular miRNAs via p53 [81], and its downstream targets are miR-34a and miR-23b, increasing cell growth and migration in cervical tissues.

miRNA	Chromosome location	Wang et al [108]	Li et al [109]	Witten et al [110]
miR-15a	13q14.2	Up		
miR-15b	3q25.33	Up	Up	
miR-16	13q14.2	Up	Up	
miR-17-5p	13q31.3	Up	Up	
miR-20a	13q31.3	Up	Up	
miR-20b	Xq26.2	Up	Up	
miR-21	17q23.1	Up	Up	Up
miR-93	7q22.1	Up	Up	
miR-106a	Xq26.2	Up	Up	
miR-146a	5q34	Up		
miR-148a	7p15.2	Up		
miR-155	21q21.3	Up		Up
miR-181c	19p13.13	Up		
miR-182	7q32.2	Up	Up	Up

miRNA	Chromosome location	Wang et al [108]	Li et al [109]	Witten et al [110]
miR-183	7q32.2	Up		
miR-185	22q11.21	Up	Up	
miR-223	Xq12	Up		
miR-224	Xq28	Up	Up	
miR-324-5p	17p13.1	Up		
miR-10b	2q31.1		Down	Down
miR-29a	7q32.3	Down	Down	
miR-30b	8q24.22	Down		
miR-34a	1p36.22	Down	Down	
miR-125a	19q13.41	Down		Down
miR-125b	11q24.1		Down	Down
miR-126	9q34.3	Down	Down	
miR-127	14q32.2	Down	Down	
miR-133a	18q11.2	Down		
miR-133b	6p12.2	Down		Down
miR-143	5q32	Down	Down	Down
miR-145	5q32	Down		
miR-191	3p21.31	Down		
miR-218	4p15.31;5q34	Down	Down	
miR-378 (422b)	5q32	Down		
miR-422a	15q22.31	Down		
miR-424	Xq26.3	Down	Down	Down
miR-450	Xq26.3	Down	Down	Down
miR-455	9q32	Down	Down	
miR-574	4p14	Down		
Up: upregulated in cervical cancer; down, downregulated in cervical cancer.[81].				

Table 2. Summary of miRNA signatures in cervical cancer.

4. Clinical utility of molecular HPV diagnosis

The incidence of cervical cancer in the decades to come might in fact increase in developing countries due to the aging of the population and the persistent absence of adequate screening programs [95]. In countries with screening, the target of preventive efforts has shifted from cervical cancer detection to the diagnosis and treatment of cancer precursor lesions. However,

the morphologic basis of the screening test cannot be substantially improved, inherently diminishing the accuracy for precursor lesion diagnosis. In these countries, a false negative Pap test occurs in 30% of all cervical cancers diagnosed and another 10% is attributable to errors in following up abnormal cytology reports [95,96].

Despite this limitation, a significant reduction in cervical cancer has been achieved with cytology-based technology and screening strategies [95]. HR-HPV DNA testing is currently recommended for triage of cytological diagnoses of ASCUS, as a cotest with the Pap smear in the general screening of women ≥ 30 years of age, and for follow-up of women after colonoscopy and treatment [97].

Histological examination of colposcopy-guided biopsies is still considered the “gold standard” in the assessment of cervical lesions; however, the histologic assessment of these lesions is limited to the interpretation of the morphology, with little to no information regarding the risk of persistence, progression, or regression. In addition, histologic assessment of cervical lesions is complicated by interobserver variability. The main interpretive categories include distinguishing normal from dysplasia (CIN) of any grade and low-grade (CIN1) lesions from high-grade (CIN2/3) lesions. Errors in histologic diagnosis lead to either overtreatment of patients who will not benefit from intervention or, conversely, undertreatment of patients with clinically significant high-grade lesions that received false negative diagnoses [98].

Contemporaneous cervical cancer screening guidelines from the American Cancer Society (ACS) and the American College of Obstetricians and Gynecologists (ACOG) in effect acknowledged the extremely high sensitivity of FDA-approved Pap and HPV co-testing by specifically accepting lengthened screening intervals for women who test negative on both cytology and HPV tests [103]. There is ample evidence that the detection of HPV DNA in cervical samples has a higher sensitivity for cervical cancer and precancerous lesions than the Pap test and high-quality HPV tests are routinely used in prevention programs in some developed countries [99].

Although the recent introduction of a highly effective prophylactic HPV vaccine has great promise for the prevention of persistent infections and precancerous lesions, cervical cancer screening will still be required because the current vaccines do not protect against all carcinogenic HPV types and do not treat preexisting HPV infections and related disease [97]. Since persistent infection with HR-HPV is a risk factor for progression to cervical cancer and with the advent of HPV vaccines, it is increasingly relevant to perform HPV genotyping to identify oncogenic HPV vaccine types. HPV genotyping is of clinical interest, since the risk of developing a precancerous lesion is between 10%, and 15% with HPV types 16 and 18, and below 3% for all other high-risk types combined. Genotyping information could provide more information regarding risk-stratification as well as persistence of infection [98].

While current guidelines and recommendations consistently advise on vaccinating young girls before their sexual debut, natural history studies indicate that all sexually active women are at risk of new oncogenic HPV infections and of development of cervical lesions and cancer throughout their lives. The reviewed data suggest that most sexually active women have the potential to benefit from HPV vaccination, with the exception of those with current infections

with both oncogenic HPV vaccine types. Women of all ages should be able to make a well-informed decision when considering HPV vaccination [99].

Screening protocols are likely to be modified taking advantage of the higher validity of HPV tests as compared to the conventional Pap smear. Many clinical trials have compared HPV DNA testing and cytology in screening scenarios and concluded that HPV test offers a greater sensitivity (in the range of 30%) and a reduced specificity (in the range of 8%) as compared to cytology. Moreover HPV tests are less demanding in terms of manpower and quality control and automated equipments are available for high throughput performance [100]. Other biomarkers are under evaluation to increase the specificity of screening programs and for the triage of HPV positive women with normal cytology. These include HPV typing, p16 INK immunostaining and others [101].

p16INK4a has been successfully deployed for the classification of HPV-related disease for several reasons: the expression of p16INK4a is directly linked to the HPV oncogenic action, since continuous expression of E7 is necessary to maintain the malignant phenotype, the expression of p16INK4a is independent of the HPV type, and therefore, genotyping does not need to be performed, and the expression of p16INK4a by cycling cells is a specific marker of HPV-E7 overexpression or other events that inactivate Rb by immunochemistry. Additionally, improving diagnostic accuracy and reproducibility, the use of p16INK4a immunohistochemistry may help in identifying CIN1 lesions that are associated with HR-HPV types; these lesions are at an increased risk for progression to high-grade dysplasia or carcinoma [98]. Thus, the clinical assessment of HPV infection uses a combination of diagnostic cytologies, such as the Pap test in association with complementary DNA test and hostp16INK4a [101].

The new guideline regarding screening for the early detection of cervical precancerous lesions and cancer was published by the ACS and American Society for Colposcopy and Cervical Pathology (ASCC) in American Journal of Clinical Pathology [102]. The new guideline includes a review of molecular screening tests and strategies, it suggest that perhaps the largest immediate gain in reducing the burden of cervical cancer incidence and mortality could be attained by increasing access to screening (regardless of the test used) among women who are currently unscreened or screened infrequently. Incorporation of HPV testing may offer advantages over what is already a successful screening strategy if utilized (ie, cytology) [103]. Incorporation of HPV testing into cervical cancer screening strategies has the potential to allow both increased disease detection and increased length of screening intervals. The recommendations are described in the Table 3.

Population	Screening Method*	Management of Screen	Results Comments
Aged <21 years (y)	No screening		HPV testing should not be used for screening or management of ASC-US in this age group
Aged 21-29 years (y)	Cytology alone every 3 y	HPV-positive ASC-US+ or cytology of LSIL or more severe: Refer to ASCCP guidelines2.	HPV testing should not be used for screening in this age group

Population	Screening Method*	Management of Screen	Results Comments
		Cytology negative or HPV-negative ASC-US†: Rescreen with cytology in 3 y	
Aged 30-65 years (y)	HPV and cytology "cotesting" every 5 y (preferred)	HPV-positive ASC-US or cytology of LSIL or more severe: Refer to ASCCP guidelines2 HPV positive, cytology negative: Option 1: 12-mo follow-up with cotesting Option 2: Test for HPV16 or HPV16/18 genotypes • If HPV16 or HPV16/18 positive: refer to colposcopy • If HPV16 or HPV16/18 negative: 12-mo follow-up with cotesting Cotest negative or HPV-negative ASC-US: Rescreen with cotesting in 5 y	Screening by HPV testing alone is not recommended for most clinical settingst.
	Cytology alone every	HPV-positive ASC-US† or cytology of LSIL or more severe: Refer to ASCCP guidelines2 Cytology negative or HPV-negative ASC-US†: Rescreen with cytology in 3 y	
Aged >65 years (y)	No screening following adequate negative prior screening		Women with a history of CIN2 or a more severe diagnosis should continue routine screening for at least 20 y
After hysterectomy	No screening		Applies to women without a cervix and without a history of CIN2 or a more severe diagnosis in the past 20 y or cervical cancer ever
HPV vaccinated	Follow age-specific recommendations (same as unvaccinated women)		

ASCCP, American Society for Colposcopy and Cervical Pathology; ASC-US, atypical squamous cells of undetermined significance; CIN2, cervical intraepithelial neoplasia grade 2; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.

* Women should not be screened annually at any age by any method.

† ASC-US cytology with secondary HPV testing for management decisions. * Modified from [102].

Table 3. Summary of Recommendations of The American Society for Colposcopy and Cervical Pathology

On the other hand, educative action to prevent cervical and non cervical cancers, which are part of basic health actions, should be considered a professional commitment to the population's quality of life and a care quality commitment, emphasizing patients' autonomy in self-care. In the study in Brazil aimed to evaluate the applicability of an educational booklet that contained information for the general population about promotion and prevention of infections and neoplastic process caused by the HPV. The authors emphasized it is necessary to promote and improve campaigns to the population about the HPV and its relations with the neoplastic process to preventive strategies [104].

Proper condom use as a primary prevention measure should remain a top priority for health officials. Campaigns with a primary aim to increase sexually transmitted infection (STI) knowledge and awareness with the intention of influencing risk perceptions amongst those sexually active, may not effectively translate into an increase in prevention behaviors. To reach the public health goal of reducing STI prevalence, barriers to engaging in STI prevention need to be addressed, including preventive strategies [105].

Education should not only be considered an extra activity, but an action that redirects practices at health promotion as a whole. Suggests that preventive knowledge about the natural history of cervical and non cervical cancer and, such as use of HPV vaccination in both sex will decrease the incidence of HPV associated cancers and has the potential to be of great significance to high-risk female and male populations, the largest group to suffer from HPV-associated cancers within this greater population [105].

In addition, molecular tools are a relatively new division of laboratory medicine that detects, characterizes, and/or quantifies nucleic acids to assist in the diagnosis of human disease. Molecular assays augment classical areas of laboratory medicine by providing additional diagnostic data either in a more expeditious manner or by providing results that would not be obtainable using standard methodologies. These methods are used for detecting infectious agents have several advantages when compared to classical approaches, because these methods generally do not require growth in culture media, like HPV, which cannot be grown in conventional cell cultures [106].

In this context, researchers benefit from having a variety of molecular diagnostic tests at their fingertips; however, the clinical laboratories in the United States have a more limited selection of FDA-approved tests for HPV. Many of the HPV diagnostic kits available in regions such as Europe and Canada have not been approved for clinical use in the United States. Meanwhile, laboratories may choose to use non-approved tests as analyte specific reagents (ASRs) or home brews, although more extensive validation is required in these cases [59].

Regardless of the technical method used, careful consideration is necessary in the evaluation of diagnostic techniques for HPV screening. Most infectious disease tests strive for the highest possible analytical sensitivity, and PCR is typically the optimal method to achieve that standard. However, the more important standard for HPV screening is not analytical sensitivity but clinical sensitivity and specificity. Clinical utility of HPV screening is based on the prediction of cervical cancer, not simply the presence of the virus. Especially in young adult populations, detecting the HPV virus has little clinical use because the vast majority of these

cases will self-resolve and never develop into cancer. The high sensitivity of PCR is thus a detriment in HPV screening, because PCR can detect even miniscule amounts of virus that may have no clinical significance [59].

There are many advantages to the PCR technology for such screening applications, including automation capabilities, turnaround time, multiplexing, sensitivity/specificity, multiple specimen types, and small-specimen volume. The major concern with the *in vitro* amplification technologies is the potential for contamination [107]. PCR require that laboratories determine a threshold of detection representing a clinically significant result. Likewise, the detection of non cancer-causing, low-risk strains of HPV has virtually no clinical utility. Knowing that a low-risk HPV strain is present does not have an impact on the clinical management of a patient with cutaneous or mucosal warts. In order to prevent superfluous laboratory testing, clinicians should also heed the ASCCP guidelines for the management of women with or without cytological abnormalities [59].

The clinical laboratory must evaluate many factors in the adoption of an appropriate HPV test, including consideration of the population being served. In underprivileged areas, for example, HPV screening tests with less than optimal clinical sensitivities and specificities may still far surpass current cervical cancer screening methods. As new data emerge from recently established HPV screening methods, researchers and clinicians will continue to strive toward the goal of early and accurate detection of cervical cancer [59].

5. Conclusion

Molecular biology techniques with different sensitivity and specificity have facilitated the characterization of the entire HPV genome, where different functional regions are identified, as a profile of their gene expression. Additionally, molecular tools have been recognized as the most appropriate method to identify and type HPV genomes because of its higher sensitivity and specificity. Although the cervical cytology for CIN has been used to reduce the incidence of mortality worldwide, molecular technologies continue to evolve for many molecular diagnostic applications. Novel strategies for detection and genotyping are important to complement the screening programmes for HPV detection in women at risk of cervical cancer.

It is also important to mention the WHO Global HPV LabNet as a WHO initiative established to support the world-wide implementation of HPV vaccines through improved laboratory standardization and quality assurance of HPV testing and typing methods to promote international comparability of results. The major methods for achieving progress towards this goal are developing international biological standards as well as preparing and validating proficiency panels to qualify methods. And finally, the incorporation of HPV testing into cervical cancer screening strategies has the potential to allow both increased disease detection and increased length of screening intervals. The recommendations are described above (Table 3) and follows the so-called “Meyer-criteria” that present scientific evidence for minimal criteria for an HPV test.

Acknowledgements

This chapter was written by the researchers of the HPV Study Group in Goiânia-GO, Brazil. The authors thank S. Quail for English support.

Author details

Angela Adamski da Silva Reis¹, Daniela de Melo e Silva², Cláudio Carlos da Silva³ and Aparecido Divino da Cruz³

1 Federal University of Goiás - Biological Sciences Institute - Department of Biochemistry and Molecular Biology, Brazil

2 Federal University of Goiás - Biological Sciences Institute - Department of General Biology, Brazil

3 Pontifical Catholic University of Goiás.- Department of Medicine and Biology, Brazil

References

- [1] Villa LL. Biology of genital human papillomavirus. *International Journal of Gynecology and Obstetrics* 2006; 94 (1) S3-S7.
- [2] Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology* 2002;55(4) 244-65.
- [3] Steenbergen RD, de Wilde J, Wilting SM, Brink AA, Snijders PJ, Meijer CJ. HPV-mediated transformation of the anogenital tract. *Journal of Clinical Virology* 2005;32 (S1) S25-33
- [4] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of Pathology* 1999;189(1)12-9.
- [5] Muñoz N, Castellsagué X, de González AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. *Vaccine* 2006; 24 (3) S3/1-10.
- [6] International Agency for Research on Cancer: Monographs on the Evaluation of Carcinogenic Risks to Humans-Human papillomaviruses. Lyon: IARC Press (90) 16-36, 2007.

- [7] Lie AK, Kristensen G. Human papillomavirus E6/E7 mRNA testing as a predictive marker for cervical carcinoma. *Expert Review of Molecular Diagnostics*'s. 2008; 8(4) 405-15.
- [8] Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *Journal of Clinical Virology* 2005; 32(1) S16-24
- [9] Molijn A, Kleter B, Quint W, van Doorn L-J. Molecular diagnosis of human papillomavirus (HPV) infections. *Journal of Clinical Virology* 2005; (32S) S43–S51.
- [10] Palefsky JM. Human Papillomavirus-Related Disease in Men: Not Just a Women's Issue. *Journal of Adolescent Health* 2010; 46(2) S12-S19.
- [11] Chaturvedi, A.K. (2010) Beyond Cervical Cancer: Burden of Other HPV-Related Cancers Among Men and Women. *Journal of Adolescent Health* 2010; (46) S20–S26.
- [12] Giuliano AR, G A, & Nyitray AG. Epidemiology and pathology of HPV disease in males. *Gynecologic Oncology* 2010; 117(2) S15-S19.
- [13] Giuliano AR, Tortolero-Luna G, Ferrer E, Burchell AN, de Sanjosé S, Kjaer SK, Muñoz N, Schiffman M. & Bosch FX. Epidemiology of Human Papillomavirus Infection in Men, Cancers other than Cervical and Benign Conditions. *Vaccine* 2008; 26(10) K17-K28.
- [14] Gillison ML. Human Papillomavirus-Related Diseases: Oropharynx Cancers and Potential Implications for Adolescent HPV Vaccination. *Journal of Adolescent Health* 2008; 43(4) S52-S60.
- [15] Castellsague X, Bosch FX, Munoz N, Meijer CJ, Shah KV, de Sanjose S. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. *The New England Journal of Medicine* 2002;346(15)1105–12.
- [16] Cuschieri KS, Cubie HA. The role of human papillomavirus testing in cervical screening. *Journal of Clinical Virology* 2005; 32(1) S34-42.
- [17] Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine* 2006; 24 (3) S3/11-25.
- [18] Miralles-Guri C, Bruni L, Cubilla AL, Castellsagué X, Bosch FX, Sanjosé S. Human papillomavirus prevalence and type distribution in penile carcinoma. *Journal of Clinical Pathology* 2009; 62 (10) 870–878.
- [19] Moody, C.A. & Laimins, L.A. Human papillomavirus oncoproteins: pathways to transformation. *Nature Reviews - Cancer* 2010; 10(8) 550-560.
- [20] Cox JT. History of the use of HPV testing in cervical screening and in the management of abnormal cervical screening results. *Journal of Clinical Virology* 2009; S1 (45) S3 S12.
- [21] Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, Muñoz N. Epidemiology and Natural History of Human Papillo-

- mavirus Infections and Type-Specific Implications in Cervical Neoplasia. In: ICO Monograph Series on HPV and Cervical Cancer: General Overview. Vaccine 2008; 26(10) K1-K16.
- [22] Muñoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV. Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England Journal of Medicine 2003; 348(6) 518–27.
- [23] Ghittoni R, Accardi R, Hasan U, Gheit T, Sylla B, Tommasino M. The biological properties of E6 and E7 oncoproteins from human papillomaviruses. Virus Genes 2010; 40(1) 1-13.
- [24] Reis AAS, Silva DM, Curado MP & da Cruz AD. Involvement of CYP1A1, GST, 72TP53 polymorphisms in the pathogenesis of thyroid nodules. Genetics and Molecular Research 2010; 9(4) 2222-2229.
- [25] Tornesello ML, Duraturo ML, Guida V, Losito S, Botti G, Pilotti S, Stefanon B, De Palo G, Buonaguro L, Buonaguro FM. Analysis of TP53 codon 72 polymorphism in HPV-positive and HPV-negative penile carcinoma. Cancer Letters 2008; 269 (1) 159–164
- [26] Almeida PS, Manoel WJ, Reis AAS, Silva ER. & Saddi VA. TP53 codon 72 polymorphism in adult soft tissue sarcomas. Genetics and Molecular Research 2008; 7(4) 1344-1352.
- [27] Koushik A, Platt RW & Franco EL. p53 codon 72 polymorphism and cervical neoplasia: a meta-analysis review. Cancer Epidemiology Biomarkers & Prevention 2004; 13(1) 11–22.
- [28] Jarienė K, Vaitkienė D, Bartusevičius A, Tvarijonavičienė E, Minkauskienė M, Nadišauskienė R, Kruminis V, Kliučinskas M. Prevalence of Human Papillomavirus Types 16, 18, and 45 in Women With Cervical Intraepithelial Changes: Associations With Colposcopic and Histological Findings. Medicina (Kaunas) 2012;48(1) 22-30.
- [29] Miller AB. Quality assurance in screening strategies: reviews. Virus Research 2002; 89:295-9.
- [30] International Agency for Research on Cancer: IARC Handbooks of Cancer Prevention Vol.10. Cervix Cancer Screening. Lyon: IARC Press; 2005.
- [31] Origoni M, Cristoforoni P, Costa S, Mariani L, Scirpa P, Lorincz A, Sideri M. HPV-DNA testing for cervical cancer precursors: from evidence to clinical practice. E cancer medical science 2012; (6) 258-273.
- [32] Katyal S, Mehrotra R. Complementary Procedures in Cervical Cancer Screening in Low Resource Settings. Journal of Obstetrics & Gynaecology of India 2011;61(4) 436-438.
- [33] Baleriola C, Millar D, Melki J, Coulston N, Altman P, Rismanto N, Rawlinson W. Comparison of a novel HPV test with the Hybrid Capture II (hcII) and a reference

- PCR method shows high specificity and positive predictive value for 13 high-risk human papillomavirus infections. *Journal of Clinical Virology* 2008; (42) 22–26.
- [34] Evander M, Edlund K, Gustafsson A, Jonsson M, Karlsson R, Rylander E, Wadell G. Human papillomavirus infection is transient in young women: a population-based cohort study. *The Journal of Infection Disease* 1995;171(4)1026–30.
- [35] de Roda Husman A-M, Walboomers JMM, van den Brule AJC, Meijer CJLM, Snijders PJF. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *Journal of General Virology* 1995;(76)1057–62.
- [36] Moscicki AB. Genital infections with human papillomavirus (HPV). *The Pediatric Infection Disease Journal*. 1998;17(7) 651–2.
- [37] Dillner J. The serological response to papillomaviruses. *Cancer Biology* 1999; (9) 423–430.
- [38] Koliopoulos G, Valasoulis G, Zilakou E. An update review on HPV testing methods for cervical neoplasia. *Expert Opinion on Medical Diagnostics* 2009; (3) 123–131.
- [39] Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M. Hybrid Capture II-based human papillomavirus detection, a sensitive test to detect in routine high-grade cervical lesions: a preliminary study on 1518 women. *British Journal of Cancer* 1999; 80(9) 1306–1311.
- [40] Munoz M, Camargo M, Soto-De Leon SC, Rojas-Villarraga A, Sanchez R, Jaimes C, Perez-Prados A, Patarroyo ME, Patarroyo MA. The diagnostic performance of classical molecular tests used for detecting human papillomavirus. *The Journal Virological Methods* 2012;185(1)32–8.
- [41] Vernick JP, Steigman CK. The HPV DNA virus hybrid capture assay: what is it--and where do we go from here? *Medical Laboratory Observer* 2003; 35(3) 8–10.
- [42] Poljak M, Kovanda A, Kocjan BJ, Seme K, Jančar N, Vrtačnik-Bokal E. The Abbott RealTime High Risk HPV test: comparative evaluation of analytical specificity and clinical sensitivity for cervical carcinoma and CIN3 lesions with the Hybrid Capture 2 HPV DNA test. *Acta Dermatoven APA* 2009;(18)94–103.
- [43] Thrall MJ, Mody DR. Clinical Human Papillomavirus Testing Modalities: Established Techniques and New Directions. *Pathology Case Reviews* 2011; 16(2) 55–61.
- [44] Venturoli S, Leo E, Nocera M, Barbieri D, Criccaa M, Costa C, Santini D, Zerbini ML. Comparison of Abbott RealTime High Risk HPV and Hybrid Capture 2 for the detection of high-risk HPV DNA in a referral population setting. *Journal of Clinical Virology* 2012; (53) 121–124.
- [45] Keegan H, Mc Inerney J, Pilkington L, Grønn P, Silva I, Karlsen F, Bolger N, Logan C, Furuberg L, O'Leary J, Martin C. Comparison of HPV detection technologies: Hybrid capture 2, PreTect HPV-Proofer and analysis of HPV DNA viral load in HPV16,

- HPV18 and HPV33 E6/E7 mRNA positive specimens. *Journal of Virology Methods* 2009;155(1) 61-6.
- [46] Gravitt PE, Coutlée F, Iftner T, Sellors JW, Quint WG, Wheeler CM. New technologies in cervical cancer screening. *Vaccine* 2008; 26 (S10) K42-52.
  - [47] Lorincz A, Anthony J. Advances in HPV detection by hybrid capture. *Papillomavirus Rep* 2001;12 145-54.
  - [48] Castle PE, Lorincz AT, Scott DR, Sherman ME, Glass AG, Rush BB, Wacholder S, Burk RD, Manos M M, Schussler JE, Macomber P, Schiffman M. Comparison between Prototype Hybrid Capture 3 and Hybrid Capture 2 Human Papillomavirus DNA Assays for Detection of High-Grade Cervical Intraepithelial Neoplasia and Cancer. *Journal of Clinical Microbiology* 2003; 41(9) 4022-4030.
  - [49] Doorbar J. Papillomavirus life cycle organization and biomarker selection. *Disease Markers* 2007; 23(4)297-313.
  - [50] Vinokurova S, Wentzensen N, Kraus I, Klaes R, Driesch C, Melsheimer P, Kisseljov F, Dürst M, Schneider A, von Knebel Doeberitz M. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. *Cancer Res.* 2008;68(1) 307-13.
  - [51] Lowe B, Fulbright A, Nazarenko I. A hybrid-capture assay to detect HPV mRNA ratios in cervical specimens. *Journal of Virology Methods* 2012 ;179(1) 142-147.
  - [52] Roberts CC, Swoyer R, Bryan JT, Taddeo FJ. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with the linear array HPVgenotyping PCR assay and influence of DNA extraction method on HPV detection. *Journal of Clinical Microbiology* 2011; 49(5) 1899-906.
  - [53] Pagliusi SR, Dillner J, Pawlita M, Quint WG, Wheeler CM, Ferguson M. Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays--an update. *Vaccine* 2006; 24(S3) 193-200.
  - [54] Iftner T, Villa LL. Chapter 12: Human papillomavirus technologies. *J Natl Cancer Inst Monogr.* 2003;(31) 80-8.
  - [55] van den Brule AJ, Snijders PJ, Gordijn RL, Bleker OP, Meijer CJ, Walboomers JM. General primer-mediated polymerase chain reaction permits the detection of sequenced and still unsequenced human papillomavirus genotypes in cervical scrapes and carcinomas. *International Journal of Cancer* 1990; 45(4) 644-9.
  - [56] Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR enzymeimmunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *Journal of Clinical Microbiology* 1997;(35)791-5.

- [57] Manos MM, Kinney WK, Hurley LB. Identifying women with cervical neoplasia: using human papillomavirus testing for equivocal Papanicolaou results. *Journal of American Medicine Association* 1999;(281)1605-1610.
- [58] Gravitt PE, Peyton CL, Apple RJ. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single hybridization, reverse line blot detection. *Journal of Clinical Microbiology* 1998;(36)3020–3027.
- [59] Arney A, Bennett KM. Molecular Diagnostics of Human Papillomavirus. *LabMedicine* 2010; (41) 523-530.
- [60] Chung MY, Kim Y-W, Bae SM, Kwon EH, Chaturvedi PK, Battogtokh G, Ahn WS. Development of a Bead-Based Multiplex Genotyping Method for Diagnostic Characterization of HPV Infection. *PLoS ONE* 2009; 7 (2) e32259. doi:10.1371
- [61] Sherman ME, Schiffman MH, Lorincz AT, Herrero R, Hutchinson ML, Bratti C. Cervical specimens collected in liquid buffer are suitable for both cytologic screening and ancillary human papillomavirus testing. *Cancer* 1997; (81) 89-97.
- [62] Rebolj M, Pribac I, Lynge E. False-positive Human Papillomavirus DNA tests in cervical screening: it is all in a definition. *European Journal of Cancer* 2011;47(2) 255-61.
- [63] Astbury K, Martin CM, Ring M, Pilkington L, Bolger N, Sheils OM, O'Leary JJ. Future molecular aspects of cervical cytology. *Current Diagnostic Pathology* 2006; (12) 104-113.
- [64] Boulet GA, Micalessi IM, Horvath CA, Benoy IH, Depuydt CE, Bogers JJ. Nucleic acid sequence-based amplification assay for human papillomavirus mRNA detection and typing: evidence for DNA amplification. *Journal of Clinical Microbiology* 2010; 48(7) 2524-2529.
- [65] Molden T, Kraus I, Karlsen F, Skomedal H, Nygård JF, Hagmar B. Comparison of human papillomavirus messenger RNA and DNA detection: a cross-sectional study of 4,136 women >30 years of age with a 2-year follow-up of high-grade squamous intra-epithelial lesion. *Cancer Epidemiology, Biomarkers & Prevention* 2005;14(2) 367-372.
- [66] Andersson E, Karrberg C, Thomas R, Blomqvist L, Zetterqvist B-M, Ryd W, Lindh Magnus, Horal Peter. Type-Specific Human Papillomavirus E6/E7 mRNA Detection by Real-Time PCR Improves Identification of Cervical Neoplasia. *Journal of Clinical Microbiology* 2011; 49(11) 3794–3799.
- [67] Clifford G M, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis *British Journal of Cancer* 2003; 89(1) 101-105.
- [68] Cushieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore C. Multiple high-risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *Journal of Clinical Pathology* 2004;(57) 68-72.

- [69] Molden T, Nygard JF, Kraus I, Karlsen F, Nygard M, Skare GB, Skomedal H, Thorensen SO, Hagmar B. Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL Pap smear. *Internacional Journal of Cancer* 2005;114(6) 973-976.
- [70] Szarewski A, Ambroisine L, Cadman L, Austin J, Ho L, Terry G. Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears. *Cancer Epidemiology, Biomarkers & Prevention* 2008; (17) 3033-3042
- [71] Ratnam Samuel, Coutlee F, Fontaine D, Bentley J, Escott N, Ghatage P, Gadag V, Holloway G, Bartellas E, Kum Nick, Giede C, Lear A Clinical Performance of the PreTect HPV-Proofer E6/E7 mRNA Assay in Comparison with That of the Hybrid Capture 2 Test for Identification of Women at Risk of Cervical Cancer. *Journal of Clinical Microbiology* 2010; 48(8) 2779–2785.
- [72] Rodriguez-Lazaro D, Lloyd J, Ikononopoulos J, Pla M, Cook N. Unexpected detection of DNA by nucleic acid sequence-based amplification technique. *Molecular and Cellular Probes* 2004; 18:251-253.
- [73] Benevolo M, Vocaturo A, Caraceni D, French D, Rosini S, Zappacosta R, Terrenato I, Ciccocioppo L, Frega A, Rossi PG. Sensitivity, Specificity, and Clinical Value of Human Papillomavirus(HPV) E6/E7 mRNA Assay as a Triage Test for Cervical Cytology and HPV DNA Test. *journal of Clinical Microbiology* 2011; 49(7) 2643-2650.
- [74] Ratnam Samuel, Coutlee Francois, Fontaine Dan, Bentley James, Escott Nicholas, Ghatage P, Gadag V, Holloway G, Bartellas E, Kum N, Giede C, Lear A. Aptima HPV E6/E7 mRNA Test Is as Sensitive as Hybrid Capture 2 Assay but More Specific at Detecting Cervical Precancer and Cancer. *Journal of Clinical Microbiology* 2011; 49(2) 557-564.
- [75] Clad A, Reuschenbach M, Weinschenk J, Grote R, Rahmsdorf J, Freudenberg N. Performance of the Aptima High-Risk Human Papillomavirus mRNA Assay in a Referral Population in Comparison with Hybrid Capture 2 and Cytology. *Journal of Clinical Microbiology* 2011; 49(3) 1071-1076.
- [76] Dockter J, Schroder A, Hill C, Guzinski L, Monsonago J, Giachetti C. Clinical performance of the APTIMA® HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. *Journal of Clinical Virology* 2009; 45(S1) S55-61.
- [77] Gulliksen A, Keegan H, Martin C, O'Leary J, Solli LA, Falang IM, Grønn P, Karlgard A, Mielnik MM, Johansen IR, Tofteberg TR, Baier T, Gransee R, Drese K, Hansen-Hagge T, Riegger L, Koltay P, Zengerle R, Karlsen F, Ausen D, Furuberg L. Towards a "Sample-In, Answer-Out" Point-of-Care Platform for Nucleic Acid Extraction and Amplification: Using an HPV E6/E7 mRNA Model System. *Journal of Oncology* 2012; 905024. doi: 10.1155/2012/905024

- [78] Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, Burge CB, Bartel DF. The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. *Science* 2005; (310) 1817-1821.
- [79] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; (120)15-20.
- [80] Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Molecular Cell* 2007; 91-105.
- [81] Zheng ZM, Wang X. Regulation of cellular miRNA expression by human papilloma-viruses. *Biochimica et Biophysica Acta* 2011; (1809) 668-677
- [82] Deftereos G, Corrie SR, Feng Q, Morihara J, Stern J, Hawes SE, Kiviat NB. Expression of Mir-21 and Mir-143 in Cervical Specimens Ranging from Histologically Normal through to Invasive Cervical Cancer. *Plos One* 2011; 6(12) 1-8.
- [83] Farazi TA, Spitzer JI, Morozov P, Tusch T. mRNAs in human cancer. *Journal of Pathology* 2011; (223) 102-115.
- [84] Cullen BR. Viruses and microRNAs. *Nature Genetics* 2006; (38) S25-S30.
- [85] McBee WC, Gardiner AS, Edwards RP, Lesnock JL, Bhargava R, Austin RM, Guido RS, Khan, S.A. MicroRNA Analysis in Human Papillomavirus (HPV)-Associated Cervical Neoplasia and Cancer. *Carcinogenesis and Mutagenesis* 2011; 2 (1) 2-9.
- [86] Lee JW, Choi CH, Choi JJ, Park YA, Kim SJ, H SY, Kim T-J, Lee J-H, Kim B-G, Bae DS. Altered MicroRNA Expression in Cervical Carcinomas. *Clinical Cancer Research* 2008; 14 2535-2542.
- [87] Li Y, Wang F, Xu J, Ye F, Shen Y, Zhou J, Lu W, Wan X, Ma D, Xie X. Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV related target genes for mir-29. *Journal of Pathology* 2011; (224) 484-495.
- [88] Lui WO, Pourmand N, Patterson BK, Fire A. Patterns of Known and novel small RNAs in human cervical cancer. *Cancer Research* 2007; 6031-6043.
- [89] Hu X, Schwartz JK, Lewis-Jr JS, Huettner PC, Rader JS, Deasy JO, Grigsby PW, Wang X. A microRNA expression signature for cervical cancer prognosis. *Cancer Research* 2010; (70) 1441-1448.
- [90] Rao Q, Shen Q, Zhou H, Peng Y, Li J, Lin Z. Aberrant microRNA expression in human cervical carcinomas. *Medicine Oncology* 2012; 29(2) 1242-1248.
- [91] Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. *Cell Research* 2008; (18)350-359

- [92] Suzuki HI, Yamagata K, Sugimoto K, Iwamoto S, Kato S, Miyazono K. Modulation of microRNA processing by p53. *Nature* 2009; (460) 529-533.
- [93] Green DR, Kroemer G. Cytoplasmatic functions of the tumour suppressor p53. *Nature* 2009; (458) 1127-1130
- [94] Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-related genes. *Nature Review Molecular Cellular Biology* 2008; (9) 402-412.
- [95] Bosch X & Harper D. Prevention strategies of cervical cancer in the HPV vaccine era. *Gynecologic Oncology*, 2006; 103 (1) 21-24.
- [96] Nirchio V, Lipsi R, Fusilli S, Ciccone E, Murino L, Santangelo A, Romano F, Di Taranto AM, Pedà D, Castriota M, Antonetti R, Bondi A. HPV infection: comparison between morphological studies and molecular biology. *Pathologica* 2008;100(3) 149-55
- [97] Marks A, Castle PE, Schiffman M, Gravitt PE. Evaluation of Any or Type-Specific Persistence of High-Risk Human Papillomavirus for Detecting Cervical Precancer. *Journal of Clinical Microbiology* 2012; 50(2) 300. DOI:10.1128/JCM.05979-11.
- [98] Hwang SJ, Shroyer KR. Biomarkers of Cervical Dysplasia and Carcinoma. *Journal of Oncology* 2012;9 doi:10.1155/2012/507286
- [99] Moy LM, Zhao FH, Li LY. Human papillomavirus testing and cervical cytology in primary screening for cervical cancer among women in rural China: comparison of sensitivity, specificity, and frequency of referral. *International Journal of Cancer* 2010; (127) 646-56.
- [100] Castellsagué X, Schneider A, Kaufmann AM, Bosch FX. HPV vaccination against cervical cancer in women above 25 years of age: key considerations and current perspectives. *Gynecologic Oncology* 2009; (115) S15-S23.
- [101] Satra M, Vamvakopoulou DN, Sioutopoulou DO, Kollia P, Kiritsaka A, Sotiriou S, Antonakopoulos G, Alexandris E, Costantoulakis P, Vamvakopoulos NC. Sequence-based genotyping HPV L1 DNA and RNA transcripts in clinical specimens. *Pathology – Research and Practice* 2009; (205) 863-869.
- [102] Saslow D, Solomon D, Lawson HW, Killackey Maureen, Kulasingam SL, Cain J, Garcia FAR, Moriarty AT, Waxman AG, Wilbur DC, Wentzensen N, Downs Jr LS, Spitzer M, Moscicki AB, Franco EL, Stoler MH, Schiffman M, Castle PE, Myers ER. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. *American Journal of Clinical Pathology* 2012; (137) 516-542.
- [103] Katki HA, Kinney WK, Fetterman B, Lorey T, Poitras NE, Cheung L, Demuth F, Schiffman M, Wacholder S, Castle PE. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. *Lancet Oncology* 2011; 12(7) 663-72.

- [104] Reis AAS, Monteiro CD, de Paula LB, da Silva RS, da Cruz AD. Human papillomavirus and public health: cervical cancer prevention. *Ciência e Saúde Coletiva* 2010; 15(1) 1060-2010.
- [105] Reis AAS, da Cruz AD, The impact of Human Papillomavirus on Cancer Risk in Penile Cancer. In: Broeck DV. (ed.) *Human Papillomavirus and Related Disease -From Bench to Bedside - A clinical Perspective*. Rijeka: In Tech; 2011. p.319-348.
- [106] Ferreira-Gonzalez A, Garrett C. Laboratory-Developed Tests in Molecular Diagnostics. In: Coleman WB and Tsongalis GJ. (2^aed.) *Molecular Diagnostics for the Clinical Laboratorian*. New Jersey: Human Press Inc; 2006. p247-256.
- [107] Voytek TM, Tsongalis GJ, Human Papillomavirus. In: Coleman WB and Tsongalis GJ. (2^aed.) *Molecular Diagnostics for the Clinical Laboratorian*. New Jersey: Human Press Inc; 2006. p447-451.
- [108] Wang X, Tang S, Le SY, Lu R, Rader JS, Meyers C, Zheng ZM. Aberrant expression of oncogenic and tumor suppressive microRNAs in cervical cancer is required for cancer cell growth. *Plos One* 2008; 3(7) e2557.
- [109] Li Y, Wang F, Xu J, Ye F, Shen Y, Zhou J, Lu W, Wan X, Ma D, Xie X. Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV related target genes for mir-29. *Journal of Pathology* 2011; 224 484-495
- [110] Witten D, Tibshirani R, Gu SG, Fire A, Lui WO. Ultra-high throughput sequencing-based small RNA discovery and discrete statistical biomarkers analysis in a collection of cervical tumors and matched controls. *BMC Biology* 2010; 8 58.
- [111] Zhang Y, Dai Y, Huang Y, Ma L, Yin Y, Tang M, Hu C. Microarray profile of micro-ribonucleic acid in tumor tissue from cervical squamous cell carcinoma without human papillomavirus *Journal of Obstetrics and Gynaecology Research* 2009; 35(5) 842-849
- [112] miRBase: the microRNA database. [http:// www.mirbase.org/](http://www.mirbase.org/) (accessed 19 August 2012).