CHAPTER 7

Methods for detection of HPV infection and its clinical utility

Luisa Lina Villa *, Lynette Denny

Abstract HPVs cannot be cultured and the detection of virus relies on a variety of techniques used in immunology, serology, and molecular biology. Currently the only FDA-approved commercially available method for the detection of HPV DNA is the Hybrid Capture assay, version hc2 (Digene, Gaithersburg, MD, USA) which is able to detect 13 high-risk types of HPV. The advantage of PCR-based methods of HPV DNA detection is that they allow for the identification of different types of HPV. This article discusses the advantages and disadvantages of the different methods of HPV DNA detection. HPV DNA testing can be used in a variety of clinical scenarios that include a primary screening test, particularly in women older than 30 years; as an adjunctive test to cytology; to triage women who have an equivocal cytologic finding, e.g., ASC-US, or for follow up post-treatment. In addition, HPV DNA testing can be performed on samples obtained by women themselves (so-called self-sampling), which may be useful in women who are resistant to undergoing gynecologic examinations.
© 2006 International Federation of Gynecology and Obstetrics. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The diagnosis of human papillomavirus (HPV) can be inferred from morphologic, serologic, and clinical findings. In productive infections, such as warts, virus particles about 50 nm in diameter can be detected by electron microscopy and by immune detection of the virus capsid proteins (L1, L2). Immunological detection of HPV in human cells or tissues has been hindered by 3 main reasons: (1) the late, capsid proteins are only expressed in productive infections; (2) the early proteins are often ex-

pressed in low amounts in infected tissues; and (3) there is a lack of sensitive and specific antibodies of high quality against the viral proteins. Antibodies generated against bovine papillomavirus (BPV) late proteins have been widely used because of their observed cross-reaction with HPV late proteins. However, they have low sensitivity and do not discriminate between HPV types, which would be essential for disease risk determination. Detection of HPV early proteins is even more complicated owing to the low expression levels generally observed in cells or tissues derived from HPV-positive lesions. Antibodies against E6 or E7 are available but their use is mostly restricted to in-vitro assays, including direct visualization of the antibodies in cells or tis-

* Corresponding author.

sues (immune histochemistry) or in protein extracts (Western blots and immune precipitation assays).

2. Assays for the detection and typing of HPVs

Because HPV cannot be propagated in tissue culture, in most cases its accurate identification relies on molecular biology techniques. With a double-stranded DNA genome of about 8000 base pairs and a well-known physical structure and gene organization, tests of choice for detecting HPV from clinical specimens are based on nucleic acid probe technology. Direct detection of HPV genomes as well as transcripts can be achieved with hybridization procedures that include Southern and Northern blots, dot blots, in-situ hybridization, signal-amplification molecular technology (Hybrid Capture assay, version hc2; Digene, Gaithersburg, MD, USA), and DNA sequencing [1]. A variety of signal detection procedures can further increase the sensitivity of these assays. The only procedure that may be capable of recognizing all HPV types and variants present in a biologic specimen is DNA sequencing of the viral genome, either after cloning into plasmids or by direct sequencing of a polymerase chain reaction (PCR) fragment. The methodology, however, is presently labor intensive and requires expensive equipment. Moreover, direct sequencing of specimens containing multiple HPV types awaits further development.

For HPV genome analysis, hybridization in solid phase, such as Southern blot for DNA molecules and Northern blot for RNA molecules, are excellent procedures that can generate information of quality, but are time consuming and require large amounts of highly purified nucleic acids. Moreover, hybridization in solid phase requires well-preserved, ideally full-size molecules, and therefore cannot be done with any biologic specimen − particularly those derived from fixed tissues in which DNA degradation is often observed. The procedure is also technically cumbersome and time consuming, and therefore not amenable for large-scale population studies. In-situ hybridization (ISH) is a technique by which specific nucleotide sequences are identified in cells or tissue sections with conserved morphology, therefore allowing the precise spatial localization of the target genomes in the biologic specimen. A great advantage of ISH, one that overcomes its relatively low analytical sensitivity, is that it can be applied to tissues that have been routinely fixed and processed. Sensitivity can be increased by combining PCR to ISH, a procedure known as in-situ PCR [2]. ISH has been used to detect messenger RNA (mRNA)

as a marker of gene expression where levels of viral proteins are low [3]. A major limitation of the method is the potential for error in HPV typing because of probe cross-hybridization. Recent improvements, however, have allowed it to become widely used for HPV DNA and RNA detection in tissues, with high sensitivity and specificity [4].

Viral DNA and RNA can also be detected by a series of PCR-based assays. A series of polymerization steps selectively amplify the viral genomes, resulting in an exponential and reproducible increase in the HPV nucleotide sequences present in the biologic specimen [1]. A summary of the characteristics and usefulness of different HPV detection assays is presented in Table 1.

Presently, the 2 assays the most widely used for the detection of genital types are the Digene Hybrid Capture assay, version hc2 [5] and PCR with generic primers [6]. Both are suitable for automated execution and reading of high-throughput testing, which is necessary in clinical settings as well as in large epidemiological studies. As discussed in detail below, the clinical utility of HPV DNA testing relies on the ability to detect HPV types that are associated with clinically relevant disease, as are the epidemiologically and biologically defined high-risk HPV types (Figure 1)

2.1. Hybrid Capture assays

The Hybrid Capture assay hc2 is based on hybridization, in a solution of long synthetic RNA probes complementary to the genomic sequence of 13 high-risk (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 low-risk (6, 11, 42, 43, 44) HPV types. The "A" probe cocktail has RNA probes to detect low-risk types of HPV and the "B" probe cocktail has RNA probes to detect the high-risk types of HPV, in 2 separate reactions. Specific HPV DNA-RNA hybrids are formed in solution and then captured by antibodies bound to the wells of a microtiter plate that recognize specific HPV DNA-RNA hybrids. The immobilized hybrids are detected by a series of reactions that generate a luminescent product that can be measured in a luminometer. The intensity of emitted light, expressed as relative light units (RLUs), is proportional to the amount of target DNA present in the specimen, providing a semiquantitative measure of the viral load.

The Hybrid Capture hc2 assay is currently available in a 96-well microplate format, is easy to perform in clinical settings, and is suitable for automation. Furthermore, the Hybrid Capture hc2 assay does not require special facilities to avoid cross-contamination because, contrary to PCR protocols, it does not rely on target amplification to achieve

Table 1 Characteristics of tests for the detection of cervical cancer and its precursors

Test	Test sensitivity/specificity for CIN 2/3 lesions and cervical cancer	
	Analytical	Clinical
Based on cell morphology		
Pap smear/tissues [a]	NA	low/high
Colposcopy [a]	NA	moderate/low
Visual inspection [a]	NA	low/low
Detection of HPV proteins		
Immunocito/histochemistry [b]	low/high	low/low
Electron microscopy [b]	low/high	low/low
Western blots [b]	low/high	low/moderate
Detection of HPV genomes		
Direct methods		
Southern blot [b,c]	moderate/high	moderate/high
In situ hybridization [b,c]	moderate/moderate	moderate/moderate
Dot blot	low/high	low/high
Signal amplification		
Hybrid Capture [d,e,f]	high/high	high/high
Target amplification		
PCR [d,e,f]	high/high	very high–high/high–moderate
Real-Time PCR [e,f]	very high/high	very high*
Detection of anti-HPV antibodies		
ELISA peptides	low/low	low/low
VLP	moderate/high	low/low
Fused E6/E7	high/moderate	low–moderate/high

Abbreviations: ELISA, enzyme-linked immunosorbent assay.
* No data were available.
[a] Limited because of their low sensitivity; highly dependent on sampling and tissue preservation; cannot type HPV.
[b] Technically cumbersome and/or time consuming.
[c] Requires DNA and tissue preservation.
[d] Less dependent on sampling; can be done in crude samples.
[e] Suitable for high-throuput testing and automation.
[f] Provides viral load information.

High-risk types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82

Low-risk types: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108

Potentially high-risk types: 26, 53, 66

Figure 1

high sensitivity. Often only the high-risk cocktail is used, which reduces time and cost. The US Food and Drug Administration recommended the cutoff value for test-positive results to be 1.0 RLU (equivalent to 1 pg of HPV DNA per 1 mL of sampling buffer).

2.2. PCR-based assays for HPV detection and typing

PCR can theoretically produce one billion 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 this problem while using PCR protocols for HPV DNA detection [1]. The sensitivity and specificity of PCR-based methods can vary, depending mainly on the primers set; the size of the PCR product; reaction conditions and performance of the DNA polymerase used in the reaction; the spectrum of HPV types amplified and ability to detect multiple types; and availability of a type-specific assay. With the latter, very high sensitivities and specificities can be achieved, though detection of a wide spectrum of HPV types has been the preferred tool to generate the attributed disease risk by HPV types (Figure 2).

The most widely used PCR protocols employ consensus primers that have the potential to detect all mucosal HPV types, and therefore are directed to a highly conserved region of the L1 gene. Among these are the single pair of consensus primers GP5/6 and its extended version GP5+/6+ [7], and the MY09/11 pair of degenerate primers and its modified ver-
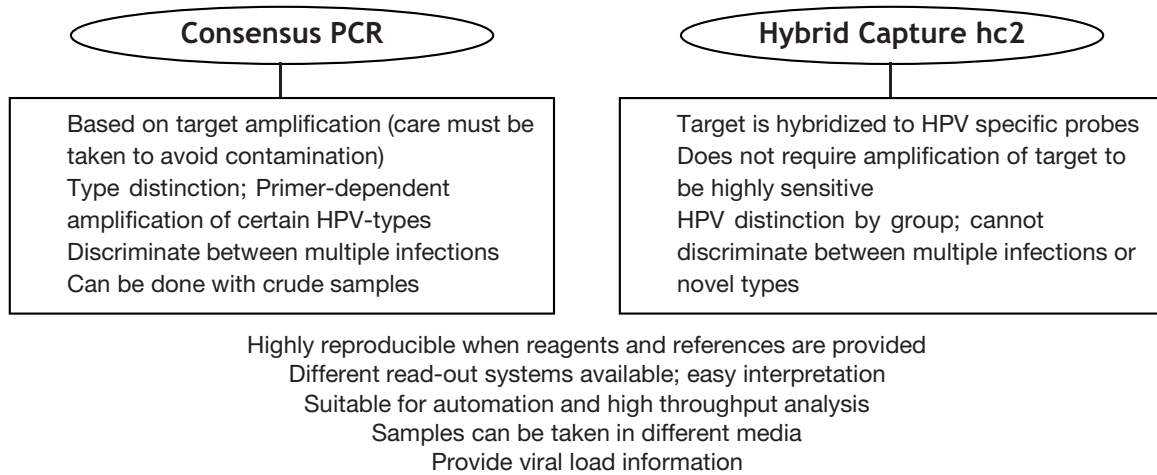
```
┌─────────────────────────┐          ┌─────────────────────────┐
│      Consensus PCR      │          │    Hybrid Capture hc2   │
└─────────────────────────┘          └─────────────────────────┘
```

Based on target amplification (care must be taken to avoid contamination) Type distinction; Primer-dependent amplification of certain HPV-types Discriminate between multiple infections Can be done with crude samples	Target is hybridized to HPV specific probes Does not require amplification of target to be highly sensitive HPV distinction by group; cannot discriminate between multiple infections or novel types

Highly reproducible when reagents and references are provided
Different read-out systems available; easy interpretation
Suitable for automation and high throughput analysis
Samples can be taken in different media
Provide viral load information

Figure 2

sion, PGMY09/11 [6]. Full distinction of more than 40 types can be achieved by hybridization with type-specific probes, which can be performed in different formats, and restriction fragment length polymorphism analysis by gel electrophoresis, dot blot hybridization, line strip assays and microtiter plates, which are amenable to automation (reviewed in [1] and [6]). Recent developments include the Amplicor Human Papillomavirus test kit (Roche Diagnostics, Calif, USA) designed to amplify with nondegenerate primers a short fragment (170 bp) of the L1 gene of 13 high-risk genotypes. A PCR-based linear array HPV product, which exploits the pGMY09/11 amplification system and is capable of identifying 37 HPV genotypes, including all high- and low-risk genotypes in the human anogenital region, is under development. Another pair of consensus primers is available that amplifies a smaller fragment of the L1 gene (65 bp, compared with 150 bp for the GP primers and 450 bp for MY09/11). This short PCR fragment (SPF)-PCR is designed to discriminate a broad spectrum of HPV types in an enzyme-linked immunosorbent assay format or reverse line blot hybridization (line probe assay) [8]. Tests that rely on shorter fragments of the viral genome are considered to be more sensitive and usable for less-preserved specimens. The SPF and GP5+/6+ systems are widely used in epidemiological studies and have been adapted to formats amenable for high-throughput testing.

Recently, PCR protocols based on a 5′-exonuclease assay and real-time detection of the accumulation of fluorescence were developed and named real-time PCR. Compared with other assays such as Hybrid Capture, real-time PCR is considered to be an accurate method of estimating viral load, while controlling for variation in the sample's cellular content by quantification of a nuclear gene. Several studies have shown the risk of developing cervical neoplasia to be associated with higher copy numbers of different HPV types [9–11]. However, inherent differences in the various assays available to determine viral load could obscure the interpretation and clinical relevance of the results obtained. Further studies to evaluate the clinical relevance of viral load are warranted.

Testing for the presence of more than 1 HPV type in the biologic specimen is preferentially done by PCR-based methods, since the Hybrid Capture assay hc2 does not discriminate between HPV types. In general, it seems that PCR systems using multiple primers such as PGMY09/11 and SPF-PCR are more robust to detect multiple infections than systems using single consensus primers such as GP5+/6+. This may especially be true in cases of mixed infections where one type is present in large amounts. Given that more accurate tools, such as the reverse line blot assays, have been developed for identifying multiple infections, it would be worth establishing whether the presence of multiple infections and lesions would be a useful marker for persistent infection and disease onset or progression.

Lately, HPV RNA has been considered an important target for molecular diagnosis of HPV infections. Unlike HPV DNA assays that detect only the presence of viral genomes, testing for viral RNA aims to evaluate the HPV genome expression (and hence viral activity in the infected cells). This is important when aiming to identify clinically relevant HPV infections. HPV-16 E6 and E7 transcripts can be detected with high sensitivity in clinical specimens by using PCR-based methodologies including reverse transcriptase PCR (RT-PCR), quantitative RT-PCR, and real-time PCR. Recent studies have shown that testing for E6/E7 transcripts of HPV types 16, 18, 31, 33, and 45 with a RNA-based real-time, nucleic acid sequence-based amplification assay (NASBA) (PreTect HPV-Proofer; Norchip, West-

fjorden, Norway), was more specific than HPV DNA detection by PCR with GP5+/6+ consensus primers in detecting high-grade cervical disease [12]. Another important application for HPV RNA studies has been suggested by Klaes and colleagues [13], who devised a method, the amplification of papillomavirus oncogene transcripts (APOT), to differentiate between episomal and integrated HPV oncogene transcripts. The rationale behind this method is that, in cervical cancers, HPV genomes are often integrated into the host chromosomes whereas in normal and premalignant tissues, viral DNA is usually kept as episomes. Using this assay, these investigators were able to show a strong correlation between detection of integrated, high-risk HPV transcripts and presence of high-grade cervical neoplasia [13]. The main problem with these techniques, however, is that RNA is a much more labile molecule that DNA, and therefore less available in most biologic specimens, depending on the time and type of storage conditions. For that reason, there is great interest in collection media capable of preserving both DNA and RNA molecules. These collection media can contain methanol, shown to preserve both cell morphology and the integrity of DNA, RNA, and proteins [1].

In general, there is good to excellent agreement rates between tests performed with the Hybrid Capture assay hc2, and with generic PCR using MY09/11 and GP5+/6+ systems. However, caution should be used when interpreting results obtained with different methodologies, because the assays differ in their ability to detect different HPV types, both in single and multiple infections. Several robust HPV tests are available, but validated protocols, reagents, and reference samples should also be available to ensure the best test performance in different settings [14]. An alternative based on the microarray technology is being exploited. HPV type-specific oligonucleotides are spotted on a glass chip. DNA obtained from the biologic specimen is submitted to a standard PCR in the presence of fluresceinated nucleotides (Cy5 or Cy3), with primers for both the β-globin (PC03/04) and the L1 region of several HPV types. Randomly labeled PCR products are then hybridized onto the chip, which is then scanned by laser fluorescence. In the case of multiple infections, multiple hybridization signals can be seen. The utility and viability of this method remains to be demonstrated.

Although the analytic sensitivities of some HPV detection assays can be very high, and therefore valuable to address the burden of HPV infections epidemiologically, its corresponding clinical significance is not so evident [15]. This is because HPV infections may not persist and therefore may not lead to clinically relevant disease. Approaches to increase the clinical sensitivity of HPV assays are being considered. These include (1) testing only for the clinically relevant, high-risk HPV types; (2) adding a viral load measure; (3) testing for high-risk HPV E6 and E7 transcripts; and (4) the natural history of HPV infections.

3. Clinical utility of HPV testing

Anogenital HPV infections are very common in young, sexually active populations [16]. In some studies, up to 70% of college-aged women are found to be HPV DNA positive [16,17]. Fortunately, most HPV infections in young women are transient, [16,18−20], and it is only the small proportion of women who become persistently infected with high-risk types of HPV who are at risk for the subsequent development of cervical intraepithelial neoplasia (CIN) 2/3 or cervical cancer. Therefore, if HPV DNA testing is to prove useful for primary cervical cancer screening, strategies need to be developed that avoid identifying large numbers of women with transient infections and focus on identifying those women with persistent infection. Transient HPV infections are much less common in women older than 30 years than among younger women, and the HPV DNA positivity rate drops considerably after the age of 30 years. Therefore, one of the easiest ways not to identify large numbers of transiently infected women is to restrict screening to women aged 30 years or older. This is not a disadvantage for most developing countries that lack the resources to screen young women. Numerous cost-effectiveness studies have clearly shown that, in settings where only 1 to 3 screens can be performed in a woman's lifetime, that screening should not be initiated before the age of 30 to 35 years.

HPV DNA testing has advantages as a screening test compared with cytologic evaluation or visual inspection of the cervix. The first advantage is its higher sensitivity, and a high sensitivity is particularly important in settings where women will be screened only once or twice in their lifetimes. The second is that HPV DNA testing not only identifies women with cervical disease but also those who are at risk for developing cervical neoplasia within the next 3 to 10 years [21]. This is particularly important for developing countries that might not have sufficient resources to screen all women at 5- to 10-year intervals, but might have the resources to screen at more frequent intervals a small subset of high-risk, HPV DNA-positive women. The final advantage of HPV DNA testing is that the interpretation of the test is objective and does not have the inherent subjectivity of visual screening methods or cervical cy-

tologic assessment. HPV DNA testing could be incorporated into screening programs in different ways, as described below.

4. HPV DNA testing as a primary screening test

A number of cross-sectional studies have evaluated the use of HPV DNA testing as a primary screening test. These studies have included significant numbers of women and have been conducted in areas of the world with a high prevalence of cervical cancer. Compared with cytologic evaluation, HPV DNA testing for high-risk types of HPV showed a consistently higher sensitivity for the detection of CIN 2/3 or greater in these studies, but it showed a somewhat lower specificity. Table 2 summarizes the findings from 4 relatively large cross-sectional studies that compared HPV DNA testing and cervical cytology.

In the context of screening, good sensitivity (i.e., the ability of the test to detect the condition of interest in all the women who have it) has to be balanced against the test's specificity. Specificity is particularly important in cervical cancer screening because screening involves large numbers of otherwise healthy women, and positive results require a follow-up colposcopic evaluation that is both uncomfortable and costly. Specificity takes on added importance in low-resource settings where colposcopy is not available, and all women who are classified as screen-positive would be treated in a "screen and treat" approach.

In a review of 14 studies, the average sensitivity and specificity of cytologic testing was 60% and 95%, respectively, while the corresponding estimates for HPV DNA testing were 85% and 84% [27]. On average, the sensitivity of HPV DNA testing is 27% higher than that of cytology in absolute terms, and its specificity is 8.4% lower. The performance of HPV DNA testing in women older than 30 years, however, was significantly better, with the average sensitivity and specificity increasing to 89% and 90%. In addition, testing for high-risk types of HPV DNA has a very high nega-

tive predictive value (NPV), that is, the likelihood of having no disease if the HPV DNA test is negative. In a number of cross-sectional studies in different populations and age groups, the NPV of HPV DNA testing was consistently greater than 97% using either the Hybrid Capture assay hc2 or PCR-based assays, with most studies reporting values greater than 99% and some reporting 100% [26].

The high NPV for high-risk types of HPV has important implications for screening programs. First, screening intervals may be significantly increased in women older than 30 years who have tested negative for high-risk HPV DNA, as the risk of these women developing cervical cancer over a 5- to 8-year period is negligible. When combining HPV DNA testing with cytologic testing, women who test negative with both methods may receive a very high level of reassurance that they will not be at risk for cervical cancer for a long time. Large cohort studies are showing that women with HPV DNA-negative cervical smears are at a very low risk for CIN 3 or cancer. The same is not true for women with HPV DNA-positive smears for high-risk types and/or a cytologic result of asymptomatic squamous cells of unknown significance (ASC-US) or higher in the classification [27,28].

The results of cytologic evaluation and HPV DNA testing will be negative in most screened women. This will be true for at least 80% of screened women, particularly in developed countries where high-risk HPV DNA is detected in less than 15% of women older than 30 years — in as few as 4% in some studies. In developing countries, rates of HPV DNA positivity in women older than 30 years appear to be higher, reaching 22% in a number of studies [24].

Another option is to use cytologic assessment to triage women who test positive for HPV DNA. Women found to be HPV positive, but with a negative or ASC-US cytologic result, could be safely managed with repeated testing 12 months later [28]. Owing to the 20% or so greater sensitivity of HPV testing, this approach would improve detection rates of high-grade cervical cancer precursors without increasing the colposcopy referral rate.

Table 2 Performance of the Digene Hybrid Capture assay, version hc2, and cervical cytologic testing for screening women aged 30 years and older in cross-sectional studies*

Country	No. of study participants	Sensitivity, %		Specificity, %	
		Cytologic testing	HPV DNA testing	Cytologic testing	HPV DNA testing
Mexico [22]	6115	57	94	99	94
Costa Rica [23]	6176	80	86	95	94
South Africa [24]	2925	74	84	88	82
Germany [25]	8466	98	37	95	99

*Digene Hybrid Capture assay, version hc2; Digene, Gaithersburg, MD, USA.

A concept similar to the one described above, known as 2-stage screening, was investigated in a South African study [29]. The concept evaluated was to screen women with a relatively nonspecific screening test (such as visual inspection or HPV testing). Only women with an initial positive test would be re-screened with a second test. Women would be referred for further evaluation (or, in a low-resource setting, for treatment) only if the results of both tests were positive. In a cross-sectional study of 2944 previously unscreened women aged between 35 and 65 years and using the first-generation Hybrid Capture assay (now referred to as hc1), screening women first for HPV DNA, and performing a cytologic evaluation only in those found to be HPV positive, resulted in a sensitivity, specificity, and NPV for HSIL and cancer of 68%, 97%, and 99%, respectively. In addition, the percentage of women referred for further investigation was dramatically reduced, from 16% for HPV testing used alone to 5% when used in a 2-stage screening algorithm.

Another aspect of HPV DNA testing is the low specificity of the test, which results in relatively large numbers of women with a positive test result without having cytologic or histologic evidence of disease. These women, however, are at a significantly higher risk of developing cervical cancer precursors and need more intensive surveillance. Castle and colleagues [30] analyzed a subcohort of 2020 women with negative results on cytologic evaluation but positive results with the Hybrid Capture hc2 assay who were followed up for a period of 57 months. These authors found that an abnormal cervical smear was found in 15% of these women within 5 years. Koutsky and colleagues [21] followed up for 25 months a group of 241 women who originally had normal cytologic results. The cumulative incidence of biopsy-confirmed, high-grade cancer precursors among the HPV-positive women in this group was 28% at 2 years, compared with 3% among those who were HIV-negative. These findings suggest that, over time, the specificity of HPV DNA testing increases significantly, and that HPV DNA testing identifies a group of women who require closer surveillance.

In addition, the low specificity of HPV DNA testing could be addressed by either limiting the types of HPV DNA detected in the assay or by raising the threshold for the definition of a positive test. Using the Hybrid Capture hc2 for HPV DNA testing in a group of women aged 35 years and older, Cuzick and coworkers [28] obtained a sensitivity higher than 95.5% using a cut-off level for a positive test of 4pg/mL. With cutoff values of 1pg/mL and 2 pg/mL, referral rates for colposcopy were 6.8% and 4.2%, respectively.

5. HPV DNA testing of self-collected vaginal samples

Wright et al. [31] published data on supervised self-testing for high-risk types of HPV DNA in a South African cohort of women. In self-collected vaginal samples the sensitivity of HPV DNA testing for CIN 2/3 or cancer was 66% (95% confidence interval [CI], 52—78%). This result was equivalent to that obtained with the conventional cervical smear when the detection of low-grade squamous intra-epithelial lesions (LSILs) or higher was defined as a positive test result (61%; 95% CI, 47—73%; P = 0.58). In contrast, the sensitivity of HPV DNA testing from clinician-obtained samples was 84% (95% CI, 71—92%), which was significantly higher than the sensitivity of a conventional cervical smear and of a sample self-collected for HPV DNA testing.

Belinson and coworkers [32] compared the sensitivity of self-sampling for HPV DNA testing with direct sampling from the cervix and liquid-based cytology (LBC) in 8497 women in Shanxi Province, China. Sensitivity for the detection of CIN 2 lesions or greater was 87.5% for self-sampling, 96.8% for direct sampling, and 88.3% for LBC if the cutoff for a positive test was defined as ASC-US or greater.

These data suggest that, in settings where cytologic screening is not available or where women are reluctant to undergo a gynecologic examination, HPV DNA testing from self-collected samples may be useful for identifying women at risk for cervical disease. The limitations of this approach, however, need to be appreciated, particularly the lower sensitivity for high-grade lesions compared with a clinician-obtained sample, and the lower specificity than that reached with cytologic assessment in some studies.

6. HPV DNA testing in the triage of equivocal (ASC-US) or low-grade (LSIL) cytologic findings

The first formally accepted clinical use of HPV DNA testing by the US Food and Drug administration (FDA) was for the triage of women found to have ASC-US. The issue raised by low-grade cytologic findings (ASC-US or LSIL) is that a number of studies have shown that, in 5% to 20% of cases, women with such findings may harbor undetected high-grade lesions. The optimum management of women with low-grade cytologic findings has thus been controversial, and options have included immediate colposcopy or repeated cytologic assessment at 6- to 12-month intervals. But both options

require repeated clinic visits and may cause women considerable anxiety.

The ASCUS/LSIL Triage Study (ALTS) trial is a large, multisite, randomized trial specifically designed to evaluate 3 methods of managing women with cytologic findings of ASC-US and LSIL [34]. The 3 methods compared were (1) immediate colposcopic examination for all women; (2) HPV testing and referral for colposcopy if the HPV test result was positive; and (3) repeated cytologic assessment with referral for colposcopy if the smear showed the presence of HSIL. Approximately 80% of the women who had a cytologic diagnosis of LSIL were found to harbor HPV DNA. The high rate of HPV positivity among women found to have LSIL on cytologic evaluation significantly undermined the ability of HPV testing to discriminate between clinically nonsignificant cytologic abnormalities and abnormalities representing true cervical cancer precursors [34]. The study concluded that HPV testing was not of value in the management women found to have LSIL on cytologic evaluation, and the American Society for Colposcopy and Cervical Pathology (ASCCP) recommend that these women undergo colposcopy instead of HPV testing [35].

With regard to the management of women found to have ASC-US on cytologic evaluation [34], the ALTS trial found that HPV testing detected 96.3% of women with previously undiagnosed CIN 3 or cancer and resulted in the referral of only 56.1% of women for colposcopy. This would significantly reduce the number of women requiring colposcopy, particularly if the management strategy is to perform colposcopy on all women found to have ASC-US on cytologic evaluation.

Consensus management guidelines for the follow-up of women with ASC-US developed under the sponsorship of the ASCCP include repeated cytologic assessment, immediate colposcopy, and HPV testing as options. However, if LBC was used for the initial cervical smear, then reflex HPV testing (that is, using the residual fluid in the LBC sample for HPV testing if the cytologic diagnosis is ASC-US) is the preferred option, as it makes a second clinic visit unnecessary [36].

7. HPV DNA testing for follow-up post treatment

Ablative or excisional techniques for the treatment of cervical cancer precursors are generally reported to be very effective, with more than 90% of "cure rates" reported. However, in approximately 5% to 15% of cases, the precursor lesions will persist or recur, requiring close follow-up and re-treatment once

lesions have been identified again [37]. In addition, treated women remain at increased risk of cervical cancer for at least 8 years compared with the general female population [38]. Traditionally, a combination of cytologic and colposcopic assessments has been used to follow up women post-treatment. Based on the fact that without detectable HPV the likelihood of post-treatment persistence or recurrence of disease is negligible, HPV DNA testing has recently been investigated as an alternative to these 2 diagnostic modalities for the detection of persistent or recurrent disease. After combining the results of 10 studies of post-treatment HPV testing, Lorincz [39] estimated the sensitivity, specificity, and NPV of HPV testing for the post-treatment detection of CIN 2/3 to be 96.5%, 77.3%, and 98.8%, respectively. Many of these studies had different designs and histologic confirmation of disease was often not available.

Paraskevaidis and associates [40], on the other hand, reported on 123 women who underwent both HPV and cytologic testing post treatment and showed the sensitivity of cytologic testing to be 48.8%, compared with 92.7% for HPV testing. The same investigators [41] also reported on 11 other published studies on the use of HPV testing after conservative treatment for CIN — i.e., excision of the transformation zone. Eight of the studies were prospective, and treatment was considered a success in 75.3% and a failure in 24.7% of the 900 women involved. While there was marked heterogeneity among the studies, this systematic review found that the sensitivity of HPV testing for detecting treatment failures reached 100% in 4 studies, but only 47% to 67% in 2 studies. The specificity of HPV DNA testing ranged from 44% to 95%.

The information gathered so far suggests HPV testing may be significantly more reliable that colposcopy and cytology, and the findings obtained from these 10 small studies, with short follow-up periods, warrant further study of HPV testing in this context.

8. Impact of infection with HIV on the utility of HPV testing as a screening test

Numerous studies have shown that the prevalence and incidence of cytologically detected SIL, biopsy-confirmed CIN, and infection of the cervix with HPV are significantly more common in HIV-infected than in HIV-uninfected women. In addition, there is evidence that in HIV-infected women HPV infection is not only more common, but more likely to be persistent and more likely to include multiple viral types. Thus, cervical pathology is common in HIV-

infected women, and the development of a rational approach to screening and the subsequent management of cervical disease is important. Hence, research is ongoing with HIV positive women treated and not treated with antiretroviral therapy.

9. Conclusion

There are data supporting the use of HPV DNA testing as a viable alternative to cytologic screening, and this is particularly important for countries that have not begun to invest resources and effort in the development of cytology-based screening programs. Prior to the widespread introduction of HPV DNA testing into clinical practice and for primary screening, a number of issues need to be addressed, including its low specificity and its high prevalence in young sexually active individuals. Currently, the cost of the most widely utilized HPV test, the Digene Hybrid Capture assay hc2, is beyond the reach of most of the countries that have not established cytology-based screening programs, but efforts are being made to develop a more affordable HPV DNA test.

References

[1] Iftner T, Villa LL. Chapter 12: Human papillomavirus technologies. J Natl Cancer Inst Mono. 2003;31:80–8.

[2] Nuovo GJ, Moritz J, Walsh LL, MacConnell, P, Koulos J. Predictive value of human papillomavirus DNA detection by filter hybridization and Polymerase Chain Reaction in women with negative results of colposcopic examination. Anatom Pathol 1992;98(5):489–92.

[3] Stoler MH, Wolinsky SM, Whitbeck A, Broker TR, Chow L. Differentiation-linked human papillomavirus types 6 and 11 transcription in genital condylomata revealed by in situ hybridization with Message-Specific RNA probes. Virology 1997;172:331–40.

[4] Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. Clin Cancer Res 2003;9:6469–75.

[5] Lorincz A, Anthony J. Advances in HPV detection by hybrid capture [letter]. Papillomavirus Rep 2001;145:54–58.

[6] Gravitt PE, Jamshidi R. Diagnosis and management of oncogenic cervical human papillomavirus infection. Infect Dis Clin North Am 2005;19(2):439–58.

[7] Jacobs MV, Snijders PJ, Voorhorst FJ, Dillner J, Forslund O, Johansson B, et al. Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison. J Clin Pathol 1999 52(7):498–503.

[8] Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999;37:2508–17.

[9] Gravitt PE, Burk RD, Lorincz A, Herrero RF, Hildesheim A, Sherman ME, et al. A comparison between Real-Time Polymerase and Hybrid Capture 2 for human papillomavirus DNA quantitation. Cancer Epidemiol Biom Prev 2003;12:477–84.

[10] Schlecht N, Trevisan A, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, et al. Viral load as a predictor of the risk of cervical intraepithelial neoplasia. Int J Cancer 2003;103:519–24.

[11] Van Duin M, Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, Nobbenhuis MA, et al. Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int J Cancer 2002;98:590–5.

[12] Molden T, Kraus I, Skomedal H, Nygard 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 intraepithelial lesion. Cancer Epidemiol Biomarkers Prev 2005;14(2):367–73.

[13] Klaes R, Woerner SM, Ridder R, Wentzensen N, Duerst M, Schneider A, et al. Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes Cancer Res 1999;59(24):6312–6.

[14] Quint WG, Pagliusi SR, Lelie N, de Villiers EM, Wheeler CM. Results of the first world health organization international collaborative study of detection of human papillomavirus DNA. J Clin Microbiol 2006;44(2):571–9.

[15] Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J Pathol 2003;201(1):1–6.

[16] Ho GY, Bierman R, Beaqrdsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423–8.

[17] Wheeler CM, Greer CE, Becker TM, Hunt WC, Anderson SM, Manos MM. Short-term fluctuations in the detection of cervical human papillomavirus DNA. Obstet Gynecol 1996;88:261–8.

[18] Hildesheim A, Schiffman MH, Gravitt PE, Glass AG, Greer CE, Zhang T, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994;169:235–240.

[19] Evander M, Edlund K, Gustafsson A, Jonsson M, Karlsson R, Rylander E, et al. Human papillomavirus infection is transient in young women: a population-based cohort study. J Infect Dis 1995;171:1026–30.

[20] Moscicki AB, Palefsky JM, Smith G, Siboshski S, Schoolnik G. Variability of human papillomavirus DNA testing in a longitudinal cohort of young women. Obstet Gynecol 1993;82:578–85.

[21] Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. N Engl J Med 1992;327(18):1272–8

[22] Salmeron J, Lazcano-Ponce E, Lorincz A, et al. Comparison of HPV-based assays with Papanicoloau smears for cervical cancer screening in Morelos State Mexico. Cancer Causes Control 2003;14(6):505–12.

[23] Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from a high-risk province in Costa Rica. JAMA 2000;283:87–93.

[24] Kuhn L, Denny L, Pollack A, Wright TC. Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. J Natl Cancer Inst 2000;92:818–25.

[25] Petry KU, Menton S, Menton M, van Loenen-Frosch F, de

Carvalho Gomes H, Holz B, et al. Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8468 patients. Br J Cancer 2003;88:1570–7.

[26] Franco EL. Primary screening of cervical cancer with human papillomavirus tests. J Natl Cancer Inst Monog 2003;31:89–96.

[27] Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, et al. Baseline cytology human papillomavirus testing and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003;95:46–52.

[28] Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet 2003;362:1871–6.

[29] Lynette Denny, Louise Kuhn, Lili Risi, Ralph M Richart, Amy Pollack, Attila Lorincz et al. Two-stage cervical cancer screening: an alternative for resource-poor settings. Am J Obstet Gynecol 2000;183:383–8

[30] Castle S, Wacholder S, Sherman ME, Lorincz AT, Glass AG, Scott DR, et al. Absolute risk of a subsequent abnormal Pap among oncogenic human papillomavirus DNA-positive cytologically negative women. Cancer 2002;95:2145–51.

[31] Wright TC Jr ,Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000;283:81–6.

[32] Belinson JL, Qiao YL, Pretorius RG, Zhang WH, Rong SD, Huang MN, et al. Province cervical cancer screening study II: self-sampling for high-risk human papillomavirus compared to direct sampling for human papillomavirus and liquid based cervical cytology. Int J Gynecol Cancer 2003;13:819–26.

[33] Schiffman M, Adrianza ME. ASCUS-LSIL Triage Study. Design methods and characteristics of trial participants. Acta Cytol 2000;124:203–11.

[34] The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomised trial. J Natl Cancer Inst 2000;92(5):397–402.

[35] Wright TC Jr, Schiffman M, Solomon D, Cox JT, Garcia F, Goldie S, et al. Interim Guidance for the use of Human Papillomavirus DNA Testing as an Adjunct to Cervical Cytology for Screening. Obstet Gynecol 2004;103:304–9.

[36] Wright TC Jr, Cox JT, Massad L, Twiggs LB, Wilkinson EJ; ASCCP-Sponsored Consensus Conference. Consensus guidelines for the management of women with cervical cytological abnormalities. JAMA 2002;287:2120–9.

[37] Mitchell MF, Tortolero-Luna G, Cook E, Whittaker L, Rhodes-Morris H, Silva E. A randomised clinical trial of cryotherapy laser vaporisation and loop electrosurgical excision for treatment of squamous intraepithelial lesions of the cervix. Obstet Gynecol 1998;92:737–44.

[38] Soutter WP, de Barros Lopes A, Fletcher A, Monaghan JM, Duncan ID, Paraskevaidis E. Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia. Lancet 1997;349:978–80.

[39] Lorincz AT. Screening for cervical cancer: new alternatives and research. Salud Publica Mex 2003;45(suppl 3): S376–S387.

[40] Paraskevaidis E, Koliopoulos G, Alamanos Y, Malamou-Mitsi V, Lolis ED, Kitchener HC. Human papillomavirus testing and the outcome of treatment for cervical intraepithelial neoplasia. Obstet Gynecol 2001;98:833–6.

[41] Paraskevaidis E, Arbyn M, Sotiriadis A, Diakomanolis E, Martin-Hirsch P, Koliopoulos G. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. Cancer Treat Rev 2004;30:205–11.