

# Understanding and incorporating human papillomavirus testing in cervical cancer screening: a South African perspective

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Keywords: human papillomavirus; HPV; cervical cancer screening; HPV DNA; HPV E6/E7 mRNA

## Abstract

The identification of human papillomavirus (HPV) as the aetiological agent for cervical cancer has important implications for the future of cervical cancer screening and prevention strategies. Despite the availability of HPV vaccines, regular and adequate screening will remain the mainstay of cervical cancer screening for some time to come. Molecular tests for high-risk HPV DNA and E6/E7 mRNA have the potential to improve cervical cancer screening in developed and developing countries. The latest international and South African private sector guidelines propose the incorporation of molecular testing for HPV in screening and patient management, backed by good scientific evidence. It is a time of transition for screening programmes; a move from the annual Pap test to a new viral paradigm. This review discusses the proper placement, advantages and disadvantages of cytology versus HPV-based screening tests from a South African perspective.

© Peer reviewed. (Submitted: 2011-03-03, Accepted: 2011-04-02)

*South Afr J Gynaecol Oncol* 2011;3(1):9-14

## Introduction

Human papillomavirus (HPV) infection is now a well-established cause of cervical cancer. It is vital that clinicians understand the benefits, limitations, and harms of the different tests that are available for cervical cancer screening. Patients must be well informed regarding the role of HPV infection testing in cervical cancer screening.

### Successes and limitations of cytological screening

Screening for cervical cancer precursors by cytology has been very successful in countries where adequate resources exist to ensure high quality of tests and good coverage of the population at risk.<sup>1</sup> Even though mortality reductions of more than 50% have been achieved in many developed countries, several limitations hamper the success in countries without appropriate infrastructure.<sup>1</sup> In a meta-analysis by Spence et al, it was found that poor cytology screening frequency was the primary factor attributable to development of invasive cervical cancer, and that almost one-third of all cervical cancer patients had previous negative cytology.<sup>2</sup>

Cytology is a subjective test and, in programmes without good quality control, it is virtually impossible to achieve and maintain clinical performance. Human errors in sampling and interpretation contribute to the poor reproducibility and low sensitivity of conventional cytology (30-87%),<sup>3</sup> and liquid-based cytology (LBC) (70-80%).<sup>4</sup> This translates into a large number of false-negative results and the need for frequent screening, which requires a high level of medical infrastructure and patient compliance. Cytology is labour intensive and is not easily adjusted to high-throughput automated screening. LBC has logistical and operational advantages, for example, interpretation at a higher speed, a lower rate of unsatisfactory smears and the possibility of additional molecular testing on the same specimen. But, LBC is also more expensive, and is neither more sensitive nor more specific than conventional cytology with respect to detection of histologically confirmed high-grade cervical intraepithelial neoplasia (CIN).<sup>5</sup> Cytology also does not have an acceptable sensitivity for the detection of cervical adenocarcinoma and its precursors.

The specificity of cytology is higher, ranging from 86-100%,<sup>3</sup> but it is anticipated that the positive predictive value (PPV) of cytological screening in

HPV-vaccinated populations will be reduced because of the expected lower prevalence of high-grade CIN among women with cytological abnormalities.<sup>1</sup> The result will be more false-positive cytology results. Thus, despite the low cost of consumables, the overall cost of high-quality cytology to the health care system may not necessarily be the most cost-effective option for screening, especially in vaccinated cohorts.<sup>6</sup>

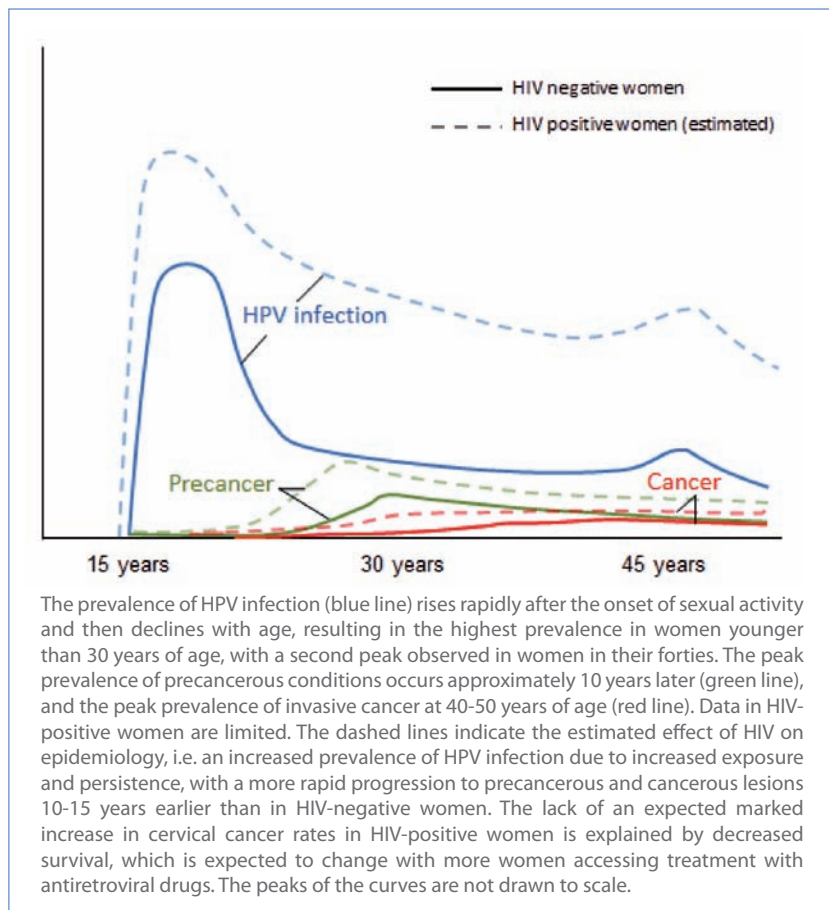
## HPV epidemiology

HPV is the most common sexually transmitted infection worldwide. Sexually active women have a life-time risk of up to 80% for infection with one or more HPV types.<sup>7</sup> HPV infection rises rapidly after the onset of sexual activity and then declines with age, resulting in the highest prevalence in women younger than 30 years of age (Figure 1).<sup>8</sup> At least 21% of women in the general South African population are estimated to harbour cervical HPV infection at any given time,<sup>9</sup> but the prevalence of high-risk HPV (hrHPV) infection may be as high as 50% in certain populations,<sup>10</sup> and 85% in women infected with human immunodeficiency virus (HIV).<sup>8</sup> Recent data suggest that the prevalence and epidemiology of HPV

infection in South Africa has increased dramatically over the last decade, due to HIV-associated immune compromise in an increasing subset of women.

HPV is found in 99.7% of cervical cancer cases,<sup>11</sup> and is also aetiologically linked to a significant proportion of anal, vulval, vaginal, penile and oropharyngeal cancers. Not all HPV types have the same ability to cause cancer; therefore, the 15 types with the highest risk have been named oncogenic or hrHPV types. Types 16 and 18 account for over 70% of cervical cancers worldwide.<sup>4</sup> Low-risk HPV types (mainly HPV6 and HPV11) cause genital warts, a proportion of low-grade cervical dysplasias, and oral, laryngeal or conjunctival papillomas.

HPV infects the actively dividing squamous cells of the basal layer when introduced through microfissures of the skin, or in naturally accessible areas like the cervical or anal transformation zones. Four out of every five genital HPV infections in immunocompetent women will be cleared by the host immune system within 18 months.<sup>12</sup> With the initial HPV infection, the viral DNA is separate from the host DNA.



**Figure 1:** The prevalence of HPV infection and cervical precancerous and cancer lesions according to age in South Africa<sup>8,9</sup>

In a small subset of women, persistent infection with hrHPV types may lead to integration of some viral DNA into the host cell DNA. If these genes include the viral oncogenes E6 and E7, uncontrolled proliferation of the cell may lead the development of premalignant cervical lesions and eventually to cervical cancer (Figures 2 and 3).<sup>13</sup> E6 and E7 mediate degradation of the tumour suppressors p53 and retinoblastoma protein (pRb) and interfere with cell-cycle regulation. E6/E7 proteins from low-risk types are less competent in interfering with p53 and pRb functions than E6/E7 proteins from high-risk types. Therefore, low-risk HPV infections are associated with benign proliferations, such as genital warts and low-grade intraepithelial lesions prone to regression. Established cofactors associated with an increased risk of persistence and integration include HIV co-infection, tobacco smoking, high parity and long-term hormonal contraceptive use.<sup>9</sup> Co-infection with *Chlamydia trachomatis* and herpes simplex virus type 2, immunosuppression, and certain dietary deficiencies have been identified as possible cofactors.<sup>9</sup>

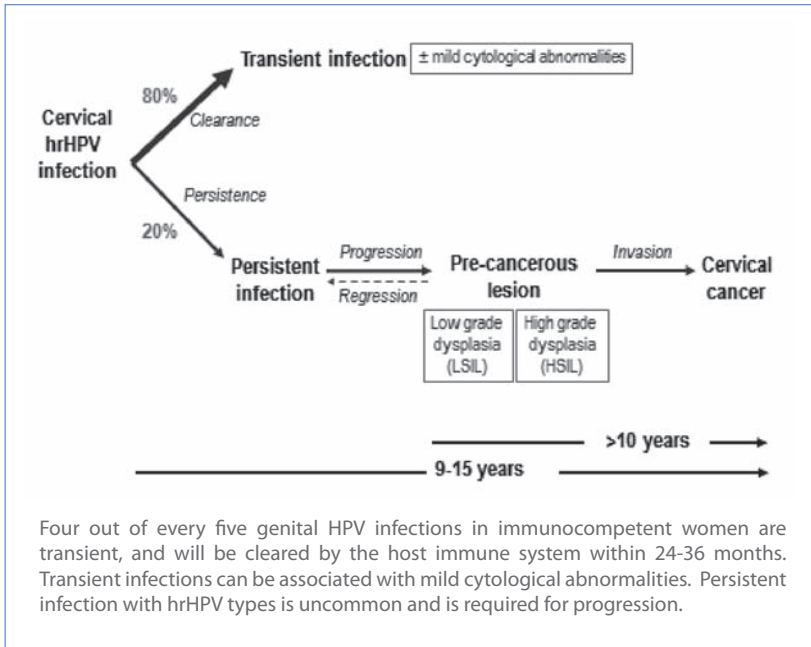


Figure 2: The natural history of HPV infection and cervical cancer<sup>14</sup>

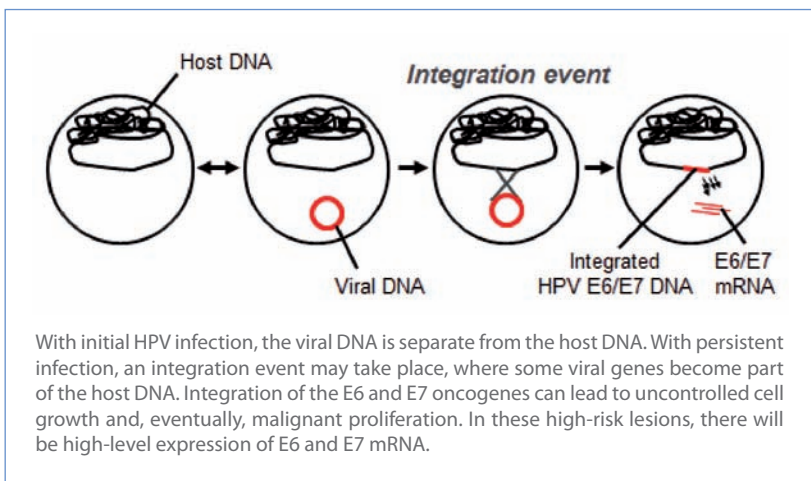


Figure 3: The steps in HPV carcinogenesis<sup>13</sup>

Cervical cancer incidence peaks in the third and fourth decade (Figure 1),<sup>15</sup> implying that it is the persistent infections occurring in women over age 30 years that are of most concern. Several studies have shown that invasive cervical cancer in HIV-positive women tends to present 10-15 years earlier than in their HIV-negative counterparts,<sup>8</sup> and this effect is not reversed by antiretroviral therapy.<sup>7</sup>

**A new approach to cervical cancer screening**

Molecular tests for HPV infection can either test for HPV DNA or messenger RNA (mRNA) of the E6/E7 oncogenes. These tests, like a Pap smear, are performed on a sample of exfoliated cervical cells, which can be obtained from LBC specimens or a cervical brush, which are placed in manufacturer-specific transport media. Protocols for

testing on patient-collected tampon specimens are in development.

**HPV DNA Testing**

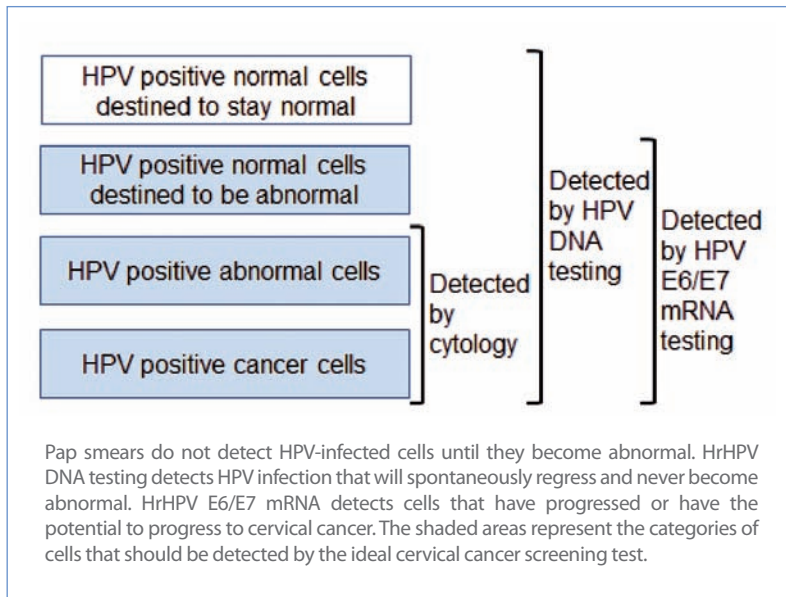
Testing for HPV DNA focuses on detection of the cause of cervical cancer, and can identify HPV-infected cells before they become cytologically abnormal. Different types of HPV DNA tests are available. Qualitative tests detect 13 to 15 hrHPV types, but do not specify which types test positive; i.e. they provide only a positive or negative result. Newer versions of these tests specify if HPV types 16 and/or 18 were present, which allows further risk stratification. HPV-genotyping assays specify 15 hrHPV types, three probable hrHPV types, and 19 low-risk HPV types.

HPV DNA testing is far more sensitive than cytology and able to detect small numbers of HPV genomes. Unfortunately, this excellent analytical sensitivity of HPV DNA testing makes it much less clinically specific. Because of this poor specificity the PPV of HPV DNA for high-grade lesions by biopsy is 15-25%, which can lead to unnecessary colposcopy and biopsy examinations in women with abnormal Pap smears who are positive for hrHPV DNA.<sup>13</sup> HPV DNA testing will also identify those women who are infected with HPV, but do not have severe dysplasia and thus have an 80% chance to clear the infection without treatment. The same positive signal is generated from infected cells

that are destined to be cleared without symptoms, to be cleared after mild dysplasia or to develop into cancer.<sup>16</sup> (See Figure 4 and Table I.)

Table I. Comparison of the results obtained from different cervical cancer screening tests for different HPV infection stages.

	Normal cell	HPV-infected cell	Transformed cell
<b>Pap smear</b>	Normal	Normal/abnormal	Abnormal
<b>HPV DNA</b>	Negative	Positive	Positive
<b>HPV E6/E7 mRNA</b>	Negative	Negative (low-level expression)	Positive (high-level expression)



**Figure 4:** The limitations of Pap smears and hrHPV DNA testing when used for cervical cancer screening<sup>13</sup>

HrHPV DNA-positive women will require follow-up and substantially more women will be referred for colposcopy and biopsy, resulting in increased costs as well as unnecessary anxiety among these women. This potential for over diagnosis of pre-invasive lesions that would have regressed spontaneously complicates the proper place of DNA testing in cervical cancer screening algorithms. Available HPV DNA-based assays differ substantially in their suitability for screening. HPV genotyping is used extensively in some areas of South Africa, with limited evidence. The use of ultrasensitive assays to detect HPV is undesirable for clinical use, because it would produce unacceptably high levels of positive results among women who would have cleared their infections without intervention. This problem is especially important in HIV-infected women with a high prevalence of hrHPV.<sup>17</sup> The cut-off sensitivity for HPV detection in newer DNA assays has been calibrated against cervical disease to an optimal clinical threshold, and promises to be more suitable for screening.<sup>17</sup> Newer tests are challenging the high cost and complexity of HPV assays. The careHPV assay (QIAGEN, Gaithersburg, MD) offers on-site testing and same-day results, which may enable “screen-and-treat” protocols to use HPV testing in low-resource settings at an affordable cost.<sup>17</sup>

The biggest advantages of HPV DNA testing are the superior sensitivity for high-grade lesions over that of cytology alone and its very good negative predictive value (~99%).<sup>16,18</sup> A woman who tests negative for hrHPV will probably not need cervical cancer screening for the next six years (range 3-10 years).<sup>19</sup> In developed countries with successful cytology-based programmes and a low HPV prevalence, a transition to HPV DNA

testing can improve efficacy and safely lengthen the screening interval in women older than 30 years of age. In populations with a high HPV prevalence, too many women will require follow-up after positive HPV DNA testing to make this assay an affordable and feasible option for screening. New technologies using HPV typing or mRNA expression may help to minimise the number of these women who would be placed on short-term follow-up schedules.<sup>1</sup>

### HPV E6/E7 mRNA testing

Several assays have been designed to detect mRNA of the E6/E7 oncogenes of hrHPV. Expression of E6/E7 mRNA increases with the severity of the lesion. In high-grade squamous interepithelial lesions (HSIL) and cervical carcinoma, high-level expression of E6/E7 mRNA is present due to the associated integration of E6 and E7 genes into the host’s cellular DNA. Expression in low-grade squamous interepithelial lesions (LSIL) is low. HPV mRNA assays have approximately the same sensitivity as HPV DNA assays, with a higher specificity and PPV for high-grade lesions.<sup>16,20,21</sup> In subjects with a high expected prevalence of disease (e.g. groups at risk, symptomatic patients, and patients with persistent cytological abnormalities after negative colposcopy results), RNA assays will provide better risk predictions than DNA tests.<sup>20</sup> HPV mRNA assays may also predict which women with LSIL or ASCUS (atypical squamous cells of undetermined significance) lesions have the potential to progress to cervical cancer (Figure 4). Possible benefits include reductions in the number of cases referred for colposcopy, improved patient well-being, and significant reductions in costs.<sup>20</sup>

### The future of cervical cancer screening in South Africa

HPV testing is the most objective and reproducible of all cervical screening tests, and is less demanding in terms of training requirements and quality assurance. It has been shown consistently to be superior to cytology in developed countries, and a recent landmark study in rural India showed unequivocally that a single round of HPV DNA testing, followed by treatment, was associated with the most significant reduction in the numbers of advanced cervical cancer and deaths from cervical cancer when compared to a single conventional cytologic test or visual inspection of the cervix with acetic acid (VIA).<sup>22</sup>

Knowing HPV prevalence patterns according to age is essential for planning a cost-effective screening

programme. HPV DNA testing should not be used for primary screening of women under 30 years of age, because HPV is a common sexually transmitted agent in young women and new infections typically resolve in this age group.<sup>22</sup> HPV mRNA testing may be a good marker to use in women younger than 30 years, because only those with a persistent transforming infection at increased risk of severe dysplasia will test positive.<sup>21</sup>

HPV screening was effective in the Indian trial,<sup>22</sup> because only 10% of the participants were found to be HPV-positive.<sup>23</sup> At a prevalence level above 20%, as in South Africa, too many women must undergo triage or treatment for a screening programme to be practical without a more specific assay.<sup>23</sup> The use of alternative HPV-based biomarkers (testing for HPV16 and other highest risk HPV types, or measuring viral E6/E7 mRNA expression) could possibly be used to implement cost-effective programmes of risk stratification in these regions.<sup>23</sup>

Some international guidelines recommend a co-testing approach, utilising HPV DNA testing and cytology in women aged 30 years and older. There is now overwhelming scientific evidence that HPV testing, as the primary screening test for women aged over 30, is better than screening with cytology. The vast majority of studies found that cytology rarely detects an HSIL that is not also HPV positive. This provides the basis for reserving cytology to triage HPV-positive women, thereby reducing the number of unnecessary referrals for transient lesions. HPV mRNA testing is now proposed as a better triage test when compared to cytology. Because HPV testing leads to a longer period of low risk than after a negative smear, adding cytology testing adds very little added benefit in terms of negative predictive value.<sup>19</sup>

HPV mRNA tests can accomplish sensitive, early-stage detection of persistent infections at risk of progression, and can also identify lesions that are likely to regress. It is possible that a single test using an mRNA assay could be more effective at detecting an hrHPV infection than repeated DNA testing.<sup>20</sup> Detection of hrHPV mRNA is considered to be potentially useful in three clinical applications:<sup>20</sup>

- As primary screening;
- In triage, to select women showing minor cytological lesions (LSIL and ASCUS) needing referral for diagnosis and treatment; and
- In follow-up of women treated for high-grade lesions, to predict persistent or recurrent disease.

Transition from conventional to HPV-based screening protocols has proven to be difficult due to high cost, limited resources in developing countries, logistical issues (e.g. lack of education of health care workers and

uncertainty regarding new HPV-related management protocols), organisational challenges (e.g. training of laboratory staff and reallocation of cytotechnologists) and education of the general public (e.g. simplification of screening demands are often negatively perceived as a reduction of acquired social rights).

### The impact of HPV vaccination on screening practices

The incorporation of HPV vaccination in developing countries and the South African public health sector is limited by the high vaccine cost. Even if HPV vaccines are affordable and widely used, they will not substantially decrease rates of cervical cancer for another five to 15 years, because of the long latency between infection and cancer.<sup>24</sup> Currently, the best secondary prevention method is regular and adequate screening.

Precancerous lesions caused by HPV16 and HPV18 will virtually disappear in vaccinated cohorts, due to very levels of vaccine efficacy. Cervical cancer screening will still be needed as a supplementary tool to prevent the 30% of cervical cancers caused by non-vaccine HPV types, and for women who were vaccinated after their sexual debut who may have been exposed to HPV before vaccination. Screening with HPV markers of disease progression at prolonged intervals will probably be the most efficient and cost effective.

### Conclusion

There is now enough scientific evidence to shift cervical cancer prevention strategies from the detection of cytological abnormalities to the detection of HPV infection markers associated with disease progression, in combination with the prevention of infection through HPV vaccination. Some critical issues regarding HPV-based screening must still be more clearly defined. These include optimal screening intervals, the best age to start screening, improved methods for management of HPV-positive patients, new biomarkers of disease progression, and optimal protocols in populations with a high prevalence of HIV.

### Conflict of interest

The author has no conflict of interest to declare.

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