

# Cervical Cancer Screening in Developing Countries

Report of a WHO consultation



Published collaboratively by  
Programme on Cancer Control,  
Department of Reproductive  
Health and Research

**World Health Organization**

# **CERVICAL CANCER SCREENING IN DEVELOPING COUNTRIES**

REPORT OF A WHO CONSULTATION



WORLD HEALTH ORGANIZATION  
GENEVA

WHO Library Cataloguing-in-Publication Data

Cervical cancer screening in developing countries : report of a WHO consultation.

1.Cervix neoplasms – diagnosis 2.Diagnostic techniques, Obstetrical and gynaecological – utilization  
3.National health programmes – organization and administration 4.Guidelines 5.Developing countries

ISBN 92 4 154572 0

(NLM/LC classification: WP 480)

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Edited and designed by Inís – [www.inis.ie](http://www.inis.ie)

Printed in France

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## PREFACE

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**C**ERVICAL CANCER is an important area of action for any cancer control programme because of the burden of disease, and the potential for effective prevention via screening.

It is the second most common cancer among women worldwide. In 2000, there were over 471 000 new cases diagnosed, and 288 000 deaths from cervical cancer worldwide. Approximately 80% of these deaths occurred in developing countries.

Cervical cancer is preventable, but most women in poorer countries do not have access to effective screening programmes.

This report documents the discussions and findings of an expert consultation called by WHO in 2001. The objectives of the meeting were:

- To develop a position paper on cytology screening in middle-income countries with specific recommendations for improving efficacy and effectiveness of programmes in this type of setting.
- To develop a status report on VIA and HPV screening for cervical cancer which analyzes level of evidence of their efficacy and effectiveness in different resource settings and highlights research issues that still need to be addressed for adequate policy development.
- To identify priority areas to be addressed by WHO with its partners.

The meeting was organized in three modules, and a chairperson was appointed for each. Dr Anthony Miller chaired the module on cytology screening, Dr Rengaswamy Sankaranarayanan the module on visual inspection with acetic acid application (VIA) and Dr Xavier Bosch the module on human papilloma virus (HPV) screening. I am extremely grateful to each of these distinguished scientists for contributing so much of their time and expertise.

For each module, key participants were chosen on the basis of their recognized expertise in the subject matter. These participants contributed actively to the considerations of their specialized subject matter, but were also able to attend the meetings of the other modules if they chose to do so. At the conclusion of the discussions on each topic the main conclusions were reported and discussed jointly by all meeting participants during the final session of the consultation. Subsequent discussions which continued for several months after the consultation helped to clarify critical issues in

each module. Again, my thanks to each of the participants in the meeting whose names are listed in Appendix 2.

The resulting report of this consultation aims to provide policy makers with the evidence base upon which to found decisions about the establishment or modification of existing cervical cancer screening programmes. It also gives insight into types of screening for which there is currently insufficient evidence on which to base a screening programme. It signals to policy makers areas that will be of importance in the future, including potentially promising screening tests such as VIA, which are the subject of current trials. If these trials yield positive results, these tests may provide effective alternatives to current screening systems.

Cervical cancer is an important public health problem, and a priority concern for the WHO Programme on Cancer Control. In its recent publication on National Cancer Control Programmes (WHO, 2002) WHO recommends early detection policies for countries with various levels of resources. Special emphasis is given to the need to develop programmes that have a systemic approach, are well integrated into the existing health system and take into account the social, cultural and economic context.

WHO will continue to monitor progress in the area of cervical cancer screening and make evidence-based recommendations about screening tests. However, the underlying truth is that irrespective of how good a screening test is, it will have no impact unless introduced as part of a well planned and implemented screening programme. It will always remain important for WHO and its Member States to work together to ensure that these systems function effectively so that the life-saving potential of cervical cancer screening can benefit women and their families in all parts of the world.

## EXECUTIVE SUMMARY

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**W**ORLDWIDE, cervical cancer comprises approximately 12% of all cancers in women. It is the second most common cancer in women worldwide but the commonest in developing countries. Cervical screening is acknowledged as currently the most effective approach for cervical cancer control. However, in many countries, including most middle-income developing countries, the existing programmes are failing to achieve a major impact.

### PROGRAMME ORGANIZATION

Central to the success of any screening programme is the functioning of that programme in its entirety. The requirements include the ability of a programme to ensure high levels of coverage of the target population, to offer high quality, caring services, to develop and monitor good referral systems that ensure good patient follow-up and to ensure that the patients receive appropriate, acceptable and caring treatment in the context of informed consent.

Cervical screening should be planned within the context of national planning for cancer control. In many countries some form of screening exists, but will have to be reorganized to achieve success. There needs to be the political will to proceed, with support and funding from the Ministry of Health. Screening has to be based on an adequate health infrastructure. There must be a defined target population, and means to identify, invite, screen and follow-up that population. The women in this population will have to be educated about screening for cervical cancer, and the health professionals who serve them may need education and retraining. A defined referral system for women with an abnormality and a mechanism to ensure women with an abnormality attend for diagnosis and treatment must be put in place. Systems to manage the abnormalities and follow-up those treated will also be required, while the programme will require monitoring and evaluation. Leadership, management skills, attention to linkages at all levels of the programme, and budgeting skills are essential.



## CYTOLOGY SCREENING IN MIDDLE-INCOME COUNTRIES

It is generally agreed that cytology screening for cancer of the cervix has been effective in reducing the incidence and mortality from the disease in many developed countries. It is the organised programmes that have shown the greatest effect, while using less resources than the unorganised programmes. There is general agreement that high quality cytology is a highly specific screening test, with estimates of the order of 98-99%. There is less agreement on the sensitivity of the test, cross-sectional studies have suggested sensitivity in the order of 50% in some circumstances. However, studies that have been able to assess sensitivity longitudinally have produced estimates that approximate to 75%.

The essential elements for successful cytology screening include:

- training of the relevant health care professionals, including smear takers, smear readers (cytotechnologists), cytopathologists, colposcopists and programme managers,
- an agreed decision on the priority age group to be screened (initially 35–54),
- adequately taken and fixed smears,
- efficient and high quality laboratory services, that should preferably be centralized,
- quality control of cytology reading,
- a means to rapidly transport smears to the laboratory,
- a mechanism to inform the women screened of the results of the test in an understandable form,
- a mechanism to ensure that women with an abnormal test result attend for management and treatment,
- an accepted definition of an abnormality to be treated, i.e. high grade lesions,
- a mechanism to follow-up treated women,
- a decision on the frequency of subsequent screens,
- a mechanism to invite women with negative smears for subsequent smears.

Elements that interfere with the development of successful cytology screening programmes include over-reliance upon maternal and child health services for screening, as women in their target group are generally too young, opportunistic rather than organised screening, and low coverage of the target group. Setting too low a threshold for referral for colposcopy, i.e. over-treating non-progressive disease, will lead to reduced cost-effectiveness.

The major advantages of cytology screening are the considerable experi-

ence accrued worldwide in its use, and that it is so far the only established screening test for cervical cancer precursors that has been shown to reduce the incidence and mortality of the disease. However, cytology has limitations, it is incompatible with some women's beliefs, and it is impossible to abolish the disease with screening. It is important that women are not coerced into screening, nor given an overoptimistic view of its potential.

New developments in cytology, such as liquid-based cytology and automated reading have advantages, but are currently out of reach of most programmes.

Research into means to improve programme efficiency in middle-income countries is a high priority.

## VISUAL INSPECTION WITH ACETIC ACID (VIA) AS AN ALTERNATIVE APPROACH TO CYTOLOGY SCREENING IN LOW-INCOME COUNTRIES

The technical and financial constraints of implementing cytology-based screening programmes in developing countries have led to the investigation of screening tests based on visual examination of the uterine cervix. Among these tests, visual inspection with 3–5% acetic acid (VIA) appears to fulfil the basic criteria of a satisfactory screening test. VIA involves non-magnified visualization of uterine cervix soaked with 3–5% acetic acid.

The results of test accuracy in cross-sectional study settings indicate that the sensitivity of VIA to detect high-grade precancerous lesions ranges from 66–96 % (median 84%); the specificity varied from 64–98% (median 82%); the positive predictive value ranged from 10–20% and the negative predictive value ranged from 92–97%. However, all reported studies, except two, suffered from verification bias. Despite different study settings, providers, study protocols and definitions of positive tests, the estimates of VIA sensitivity cluster around a mean value of 76%. In most of the studies where cytology and VIA have been provided under the same conditions, the sensitivity of VIA was found to be similar to that of cytology, whereas its specificity was consistently lower.

A wide range of personnel ranging from doctors, nurses and other allied health workers to non-medical personnel has been involved in the administration and reporting of VIA results. The most common form of reporting involved negative and positive categories. The emerging consensus is that well-defined, demarcated, densely opaque acetowhite lesions located in the transformation zone (TZ) close to the squamocolumnar junction should define a positive VIA test. The criteria for a negative test have included one

or more of: no acetowhite lesions, faint ill-defined translucent acetowhite lesions, endocervical polyps, nabothian cysts, dot-like acetowhite lesions and a prominent squamocolumnar junction.

To date, the investigation of women with a positive VIA has followed similar principles to those of cytology-positive women. In various settings, five options have been offered:

- Referral for colposcopy with histological sampling and treatment based on the histological finding;
- Referral for colposcopy with histological sampling and treatment given on the basis of the colposcopic diagnosis (with retrospective access to histological diagnosis);
- Referral for magnified visual inspection (VIAM) with histological sampling and immediate treatment with cryotherapy;
- Referral for colposcopy and treatment on the basis of the colposcopic diagnosis;
- Referral for immediate treatment with cryotherapy on the basis of a positive VIA test.

All of the above approaches are still being evaluated in terms of safety, acceptability to women, feasibility and effectiveness in eradicating pre-invasive cervical disease.

In most of the reported study settings, training in the administration and reporting of VIA has been carried out in sessions lasting 3 days to 2 weeks, accompanied by written manuals. A learning period has been recognized following the training sessions. In both reported and unreported studies, the screen-positive rate among newly trained screeners has ranged from 25–35%, which later decreased to 10–18% in most instances.

The major limitations of VIA include: low specificity (generally less than 85%), which can lead to over-investigation and over-treatment of screen positive women and lack of standardized methods of quality control, training and competency evaluation. Furthermore, it is limited in its ability to detect endocervical disease. The major strengths of VIA include its simplicity and low cost, real time availability of results and potential for immediate linkage with investigations/treatment, consistent estimates of accuracy, feasibility to be offered in low resource settings and the possibility of rapid training of providers.

Further research in addressing methods for improving specificity/reducing false positivity, quality control, tests to be used to follow-up women who have been treated and competency and evaluation of skills of screeners and other health personnel involved in screening programs is essential. The efficacy and cost effectiveness of VIA-based population-screening programmes in reducing the incidence of, and mortality from, cervical cancer is not

known and remains to be established, as do the long term complications and safety of over-treatment in the context of a VIA screening programme.

Further information from ongoing studies regarding VIA's longitudinal (programme) sensitivity, efficacy in reducing incidence/mortality from cervical cancer, its cost-effectiveness and safety will be useful in formulating public health policies to guide the organization of VIA-based mass population-based screening programmes in developing countries and to reorganize programmes in countries with current inefficient cytology screening programmes.

## HPV TESTS IN CERVICAL CANCER SCREENING PROGRAMMES: POSSIBLE ROLE IN MIDDLE-INCOME COUNTRIES

Molecular and epidemiological studies have unequivocally shown that the vast majority of cervical cancer cases worldwide are caused by persistent infections with some high-risk types of the human papillomavirus family. From the perspective of defining preventive strategies, the HPV-attributable fraction should be considered to be 100%.

Current HPV testing systems are able to detect the presence of viral markers (HPV-DNA in exfoliated cervical cells) in close to 100% of invasive cervical cancer specimens, 75 to 90% of precursor lesions (LSIL / CIN1, CIN2/3, HSIL) and in 50% of borderline cytology lesions (ASCUS).

Commercially available, FDA approved, testing systems can be transferred to laboratory settings with some level of sophisticated technology which are generally found in middle-income countries.

In triage studies (investigations of the minor abnormalities detected by cytology) and in screening studies (when both cytology and HPV tests are jointly performed) the cross-sectional sensitivity of the HPV test to detect HSIL or more advanced lesions is at least as good as cytology. In most studies, the reported sensitivity of the HPV test is some 10% higher than cytology.

Triage studies, including large randomized controlled trials, have shown that reduction in the number of visits and referrals to colposcopy/biopsy can be achieved with HPV tests.

One of the strongest gains of the combination of HPV tests and cytology lies in the very high negative predictive value (i.e. >97%). Major savings to the health systems may derive from substantially increasing the duration of the interval between screens without losses in sensitivity for high-grade intraepithelial lesions.

The advantages of HPV tests as compared to cytology are:

- The objectivity of the test resulting in very low inter- and intra-observer variability.
- The possibility of almost complete automation of the process. This should ensure high throughput at a standard level of quality.
- Built-in quality control procedures.
- Opportunities for self-sampling for HPV DNA in some populations with limitations in health care facilities and manpower, albeit with some loss of sensitivity.
- The high sensitivity of the HPV DNA test to identify HSIL in women aged 30 and above.
- Gains in effectiveness could be achieved by increasing the length of the interval between screens and reducing the total number of lifetime screens required.

The disadvantages of HPV DNA testing are:

- Cost.
- Dependence on reagents currently produced by only a single commercial manufacturer.
- The requirement for a molecular diagnostic laboratory.
- Its low specificity in younger women and populations with significant rates of HIV seropositivity.
- Furthermore, since HPV DNA testing, like cytology, is not a test that provides results at the time of the visit or soon afterwards, many of the traditional barriers to cytological screening have not been eliminated.

Cost-benefit analyses of HPV testing are underway. Modelling, based upon results from South Africa, suggests that VIA or HPV DNA tests may offer attractive alternatives to cytology-based screening programmes.

In countries with established cytology-based screening programmes, HPV tests are an alternative to repeat cytology in the presence of abnormal cytology. In countries without established cytology-based screening programmes, but with the necessary laboratory facilities, HPV tests could be evaluated for primary screening. Appropriate trials are strongly encouraged.

## INTRODUCTION

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*The aims of the consultation were:*

- to develop a position paper on cytology screening in middle-income countries, with specific recommendations for improving the efficacy and effectiveness of programmes in this type of setting;
- to develop a status report on visual inspection with acetic acid (VIA) and human papilloma virus (HPV) DNA testing with attention to their efficacy and effectiveness in detecting cervical neoplasia in different resource settings;
- to identify research issues in relation to screening with VIA and HPV testing, that need to be addressed for adequate policy development.

For the purpose of this report, the generic term “middle-income countries” embraces a variety of developing countries, some having limited access to cytology-based screening activities. Typically, these have low population coverage of screening, predominance of clinical services for women presenting with symptoms, absence of pre-established calls for screening to women in pre-defined age groups, insufficient quality control of cytology and limited follow-up of women with positive smears. In many countries this is associated with limited access to treatment, especially for precancerous lesions. Programmes tend to be decentralized and only partially funded, and organized to meet immediate needs rather than long-term follow-up and management. In these populations, combinations of health care systems with private and public practice, different modes of reimbursement for services and predominance of case-finding activities tend to occur. There are thus many different scenarios that may have in common the need of either starting *de novo* screening programmes or considerably reorganizing existing ones.



# EPIDEMIOLOGICAL STATUS OF CERVICAL CANCER

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Worldwide, cervical cancer comprises approximately 12% of all cancers in women. It is the second most common cancer in women worldwide, but the commonest in developing countries. Annual global estimates around the year 2000 are for 470 600 new cases and 233 400 deaths from cervical cancer annually (1). Eighty percent of these cases occur in developing countries.

In most countries in North America and Western Europe, the incidence of cervical cancer has been falling, although recently at a much slower rate (2). In many developing countries, however, cancer of the cervix has changed little in incidence, except for those countries that have achieved the demographic (epidemiological) transition with increasing affluence from industrialization. In such countries, there has been a fall in incidence of cancer of the cervix, and a rise in incidence in cancer of the breast, similar to changes that occurred in North America and Western Europe in the early part of the last century. Many of the countries that have been through this transition are in the “middle-income” category.

It has been estimated that the number of prevalent cervical cancer cases diagnosed in the previous five years was around 1 401 400 in the year 2000 compared with 3 860 300 for breast cancer, with 1 064 000 and 1 522 000 of these occurring in developing countries, respectively (1). Thus although breast cancer is increasing in importance in many developing countries, cervical cancer remains a major cause of morbidity and mortality.

Data are available internationally on trends and incidence of cancer of the cervix and, with some notable exceptions, tend to show declines (3). This is true for nearly all registries in the Americas, Asia, Australasia and Hawaii, and Europe. The reductions have been quite striking in Hawaii, Denmark, Finland, Sweden, Japan, and more recently in the Maoris of New Zealand but also in Cali, Colombia and Puerto Rico. In Cali, Colombia, screening programmes have been operational, and a case-control study confirmed that screened women had a reduced risk of disease (4). However, since overall coverage does not sufficiently explain all of this incidence reduction, much of it may reflect epidemiological transition. Reductions have been quite small recently in many countries with low incidence in the early 1960's including Canada, many parts of the United States, and the Caucasian population of New Zealand. In Finland there has been some recent increase in incidence, but not in mortality, in women aged 25–54 (5).





## PROGRAMME ORGANIZATION

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# 2

Central to the success of any screening programme is the functioning of that programme in its entirety. The requirements include the ability of a programme:

- to ensure high levels of coverage of the target population to be screened (80% or more);
- to offer high quality, caring services;
- to develop and monitor good referral systems that ensure good patient follow-up;
- to ensure that the patients receive appropriate, acceptable and caring management in the context of informed consent.

Programmes need to be locally appropriate and those designing the overall programme need to be aware of the multiple barriers that women may experience in accessing services (e.g. physical access, economic considerations, control over decision-making at the household level, access to information etc.) and attempt to decrease such barriers. Most tests used in cervical screening are uncomfortable and potentially embarrassing, as they require a vaginal examination with a speculum. Adequate training of health care providers on the screening programme itself, technical skills in relation to the screening technique, referral systems, treatment protocols, quality control of the screening test, etc. are essential to setting up or reorganizing screening programmes. The type of screening test is subordinate to the need to have these systems in place and functioning well. Furthermore, these systems are required, irrespective of which method of screening is used.

### THE ESSENTIAL ELEMENTS FOR SUCCESSFUL CERVICAL SCREENING

#### **An informed decision to initiate cervical screening in the context of a National Cancer Control Programme**

Cervical screening should be planned within the context of national plan-

ning, and a decision made after a determination of the relative priority of cervical cancer in the country (6).

In many middle-income developing countries cervical screening exists in some form or other, often associated with maternal or child health services, and/or as a component of private health care for affluent women. As already indicated, such programmes tend to be ineffective, as adequate coverage does not extend to the majority of women at high risk of the disease. Moreover, they are decentralized and only partially funded. Increasingly in such countries, low-income and middle-income families are struggling to provide the monies necessary for long-term care. However, it is unlikely that simply providing funds for the expansion of such efforts will enable the programme to be successful. Experience in the United Kingdom confirms that a radical reorganization of the programme is required, with appropriate incentives to ensure that relevant health care providers participate (7). In several other Western European countries with liberal health care systems, screening is often performed on an opportunistic basis, characterized by over-screening of those at low risk in conjunction with under-screening of certain social groups at high risk, and heterogeneous quality of screening. It is often impossible to document effectiveness of screening because of lack of adequate monitoring (8).

### **The political will to proceed, with support and funding from the Ministry of Health**

Although programmes can initially be based on support from external donors, they will never become self-sustaining without a political decision to support the programme and maintain it with governmental funding after the external support ends. Further, without political support, it may be extremely difficult to cope with various internal, or sometimes external, pressures to change decisions taken with regard to the programme.

### **An adequate health care infrastructure**

It is impossible to organize screening programmes in the absence of a health care system that is capable of providing the diagnostic and treatment services inseparable from screening. It is important to ensure that the introduction of screening does not adversely affect other important health services. Therefore, implementation of cervical screening should be planned so that it is consistent with the developing health care infrastructure of the country. Developing a cervical screening programme can facilitate the improved functioning of a health care system. It should, therefore, be undertaken in a manner that is integrated with existing services so as to improve health

system functioning. Health care systems can be reformed, but the appropriate advice must be given. Adequately trained managers that have the skill base to see the interrelated aspects of setting up a programme and address each are often lacking within health systems. The most profound need is support for the management functions to enable screening to be undertaken.

### **Definition of the target population**

The target population should be defined in terms of age. Rarely are other parameters appropriate, though the epidemiology of the disease in the country will guide the decision-making process (9). It is important that decisions on age are taken on the basis of age-related incidence rates of invasive cervical cancer and not on the percentage distribution by age of clinically detected cases of cancer in the country.

### **Education of the target population**

It has been well documented that professional and public education, combined with the availability of treatment for early stages of invasive cancer of the cervix, had an important effect in reducing the morbidity and mortality from the disease, long before screening programmes were introduced (10). Education is thus a basic measure that will contribute to early diagnosis of the disease, and upon which screening must be based.

It is important that the educational programmes be designed for the culture of the country, and that they observe the myths that tend to be prevalent about cancer. In some cultures men will also need information. Women should not be coerced into screening, nor should they be given over-optimistic messages concerning benefits. Thus, women should understand that a negative test, though encouraging, does not guarantee absence of disease now or in the future, and conversely, that a positive test does not mean cancer, but the need for further investigation. Education through the media will be only partially effective. Education will also be necessary at the time of administering screening tests or referring women for diagnosis. Such education must be administered by individuals who can achieve personal interaction with subjects, and should be interactive, not simply passive.

### **A means to identify the target population**

The ideal means is a population register, but this will often be unavailable, or inaccessible to the screening programme, even if present. Other means are often found to exist and be accessible, however, providing data protection

legislation permits such access. These include a locally performed census, voting registers, and existing medical records.

### **A means to invite the target population for screening**

This also will require approaches designed for local circumstances. They can include letters of invitation in literate communities, mass media invitations to attend, special efforts for recruitment with health care workers or volunteers working in the community, or utilizing contacts women make with the health care system for other purposes, if none of the other approaches are possible. In many countries it will be necessary to ensure that the woman's personal health concerns are taken care of to ensure her collaboration with screening.

### **Training of relevant health care professionals**

There are many types of health care professionals that will require training. For administration of the screening test these tend to be specific to the test that will be used, and are discussed further in the sections that follow. However, for all tests, adequately trained professionals will be required for the diagnosis and treatment of the detected abnormalities.

Initiating programmes by screening current health staff as the first step may increase empathy in service providers. From a managerial point of view a method of ongoing monitoring that tracks issues such as privacy, respectful interactions, informed consent, etc. would be important in reinforcing their centrality to service provision and to sustaining respectful caring service provision. Methods of engaging service users in monitoring the quality of care are an option that has proved useful in other programmes and should also be investigated (11).

Means must be introduced to facilitate problem solving and team learning. The problem is often to change the entrenched practices of physicians, midwives and other professionals who had been involved with screening, in spite of scarce financial resources. The methodology requires a combination of the problem identification and solving approach and a variant of the problem-based approach to medical education.

The main areas of intervention are personal (consistency, optimism, flexibility, good interpersonal relations and quality), and collective focus on "achieving" specific objectives in a systemic overview, critical mass, team work, efficient use of resources, a project planned with creative input from the local team, and quality information that is properly circulated.

The process includes:

- Definition of the problem as a quantified discrepancy between an actual and a desired situation, with the best available information and/or estimate.

- General options for solving the problem defined.
- Analysis of the options for solving the problem.
- Choice of the best solutions in light of the relevant criteria.
- Design of strategies to implement the solutions.
- Implementation of the solution.
- Review and modification of the solution in light of experience.
- Evaluation of the results.

### **A defined referral system for women with an abnormality and a mechanism to ensure women with an abnormality attend for diagnosis and treatment**

It is essential to ensure that women with a defined abnormality receive appropriate diagnostic tests, and if confirmed to have a lesion that requires treatment, receive the relevant therapy. There should be no financial barrier to such referral and attendance. Health care practitioners working at the primary level should understand the process.

Several studies have identified poor communication and feedback systems between clinic and laboratory staff and between screening centres and treatment facilities. Secondary and tertiary levels of care do not see themselves as part of a system. They see little value in reporting patient outcome and primary care sites may have difficulty making appointments for patient follow-up. This is a barrier that patients are often left to negotiate on their own. Frequently, patients referred to secondary level care sites who have abnormal results are subjected to a repeat Pap smear and are asked to return again once that result is available.

All this is compounded by poor information and monitoring systems. In many centres it is not possible to link data and thus track if a patient has presented for and received care. This again leads to de-motivated primary care staff and inadequate patient follow-up.

### **Patient management guidelines**

It is important that protocols are developed for primary care staff on how to interpret and act on screening test results. Their absence can lead to inadequate action and the risk that patients who require either repeat tests or investigation and definitive treatment will be overlooked. It also results in significant costs to the health care services and individual women when women are unnecessarily requested to present for a repeat test.

Cryotherapy, loop electrosurgical excision procedure (LEEP), laser ablation and cold knife conization of the cervix are different standard therapeutic options for the treatment of precancerous lesions and most of these can be

provided as outpatient procedures. It has now been established that all of the standard outpatient treatments for dysplasia under colposcopic guidance are highly effective and are associated with low rates of complications. No significant differences in overall failure and complication rates between these different treatment modalities have been observed in randomized clinical trials in developed countries (12–17).

It is imperative that adequate resources be identified and invested to establish a certain minimum infrastructure in terms of diagnostic (colposcopy, histopathology) and treatment (cryosurgery, LEEP, cold knife excision procedures) facilities in health services before decisions on implementing screening programmes are taken.

### Follow-up of patients

Systems to routinely follow-up patients must be in place. Such systems may be absent because of the poor introduction of the screening programmes, in part because funding for health services is decreasing and patient follow-up for any disease is not seen as a priority. Supervisors must be encouraged to see this as part of a system to improve quality of care.

### A means to identify failures of the programme, e.g. invasive cancer

If the programme is set within an area with an existing population-based cancer registry, invasive cancers will be identified by linking the screening file with the cancer registry. If a registry does not exist, a means should be found to set up a registry of all cases of invasive cervical cancer, however diagnosed, to specifically serve the programme. With both approaches, attempts should be made to distinguish cases diagnosed as a result of screening (often micro-invasive or early stage) from those that are clinically diagnosed, the true failures of the programme. However, in addition, it should be determined whether the latter cases have been previously screened, and thus are failures of the screening process, or were never screened, being failures of the recruitment process.

It is important to recognize that even when a screening programme is well organized, there will continue to be adequately screened women with true negative slides on review who develop cervical cancer. One reason could be that the lesion did not exfoliate, another that the particular malignancy progressed too rapidly for detection by screening. Thus some invasive cancers in the screened population should be expected although organization will ensure they are minimized. The population being invited for screening should also be aware of this and advised to report symptoms when they occur.

## **A defined evaluation plan**

Strategies for evaluation have been proposed (6,9,18,19). Eventually, the success of the programme will be determined by reduction in the incidence of invasive cancer. If excellent coverage with screening is achieved, incidence will fall within 10 years of starting the programme. Several intermediate endpoints can be proposed to monitor the programme:

### *Process measures:*

- >80% of women aged 35-59 years informed about screening for cancer of the cervix.
- >80% of primary health care workers instructed in screening.

### *Impact measure:*

- >80% women aged 35-39 screened at least once.

### *Outcome measure:*

- >30% reduction in proportion of cases of invasive cervical cancer with advanced (Stage II+) disease.

However, care must be taken to not over-rely on the process and impact measures, unless it can be documented that the women who enter the programme include those at high risk of the disease.

## **FUNDAMENTAL COMPONENTS ESSENTIAL FOR AN ORGANIZED PROGRAMME**

### **Leadership**

Effective leadership is critical. The leader should have characteristics, experience and qualifications similar to those defined for National Cancer Control Programmes coordinators (6).

### **Management**

Management of all phases of the programme is also critical. Programmes often fail because an important component has failed (for example, failure to ensure women with an abnormality attend for diagnosis and treatment).



Managers require training, so that they understand the requirements for effective programmes.

### **Attention to, and linkages between, all programme levels**

There are several different “levels” in a programme. For example, the level of recruitment, the level of the laboratory, the level of colposcopy, the level of treatment, the level of follow-up. The organization of the programme should ensure adequate linkages between these different levels, and ensure that each level understands what happens at the next, and there is adequate communication between them. The objective is to ensure that women proceed from one level to the next (if necessary), without the woman having to take the initiative with her limited understanding of the process.

### **Budgeting**

Each component of the programme requires realistic budgeting. The process should be conducted in collaboration with experts in the Ministry of Health. They will need specific information on the needs for each component of the programme. A detailed flow-chart will facilitate this process.

# CYTOLOGY SCREENING IN MIDDLE-INCOME COUNTRIES

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# 3

## CERVICAL CYTOLOGY AS AN EFFECTIVE SCREENING TEST

Even though the efficacy of cytology screening has never been proven through randomized trials, it is generally agreed that it has been effective in reducing the incidence of and mortality from the disease in developed countries (20-24). It is the organized programmes that have shown the greatest effect, while using fewer resources than the unorganized programmes (23,25). However, in all countries that contemplate introducing screening, this should be set within the context of National Cancer Control Programme (NCCP) planning (6), and with full attention to programmatic issues (26).

Data from the WHO cancer mortality data bank confirm major reductions in cervix cancer mortality in the Nordic countries that initiated organized programmes in the 1960s, and in Canada and the US where major efforts were made to encourage screening in the 1960s, though as yet organized programmes are not in place in North America. In the United Kingdom a major effort was begun in 1988 to initiate organized programmes, and a significant reduction in cervix cancer mortality is now being seen (27). The failure of opportunistic screening in Norway and the UK before 1988 is exemplified by the contrast between Norway and Finland and between the UK and North America in the 1980s and 1990s. In contrast, in most developing countries screening appears to have had little or no effect (28,29), with the exception of the programme in Chile (30).

## THE VALIDITY OF CERVICAL CYTOLOGY AS A SCREENING TEST

There is general agreement that high quality cytology is a highly specific screening test, with estimates of the order of 95–99%. Recently, controversy has arisen over whether cytology is sufficiently sensitive, largely because of cross-sectional studies conducted in different settings, meta-analyses of several studies (31-33) and comparisons made with VIA and HPV testing (see later sections). Estimates of cytology sensitivity of the order of 50% or even

less have been made. However, several of these studies evaluated cytology cross-sectionally as a diagnostic test, rather than as a screening test.

Sensitivity is defined as the ability of a screening test to detect all those with the disease in the detectable pre-clinical phase in the screened population, at the time the test is administered. It is usual in measuring the sensitivity of a screening test for cancer to regard the disease as the cancer itself. However, in the context of a test designed to detect precursors of a disease, the false negatives that truly matter may be difficult to determine. This reflects the fact that as the extent to which precursors destined to progress to invasive cervical cancer were missed by screening cannot be determined in a cross-sectional study, but requires prolonged follow-up and evaluation (i.e. a longitudinal study). This basic principle (34), was endorsed in a recent WHO consultation on the principles of screening (35), and therefore was not reconsidered in the present consultation. The recent meta-analyses assessed sensitivity of cytology cross-sectionally, and with few exceptions, included studies that suffered from verification bias (36), which occurs when only those who test positive are submitted to the gold standard test (often colposcopy with histologically confirmed diagnoses), such that false negatives to the screening test were simply not identified. Such a design cannot distinguish between those precursor lesions destined to regress and those destined to progress. It is the latter that need to be considered in estimating sensitivity relevant to programme planning. It is entirely conceivable that the sensitivity of tests that identify different spectra of precursors may be very different with regard to the proportion of potentially progressive lesions found. Only a few studies have assessed sensitivity of cytology longitudinally, using cancer as the endpoint. All were conducted several years ago in developed countries with high quality laboratories and produced estimates of sensitivity ranging from 60% to 90% (37,38).

In many cytology laboratories in developing countries, it is probable that the sensitivity achieved is substantially less than in these published reports (37,38). Poor sensitivity in the laboratory will be compounded if adequate smears are not taken, as there are two components of false negatives, that caused by poor smear-taking, and that caused by laboratory (processing/reader) error (37). Cytology also suffers from relatively low reproducibility (39-41). To reduce the impact of these deficiencies, there are many essential elements for successful cytology-based screening programmes.

## THE ESSENTIAL ELEMENTS FOR SUCCESSFUL CYTOLOGY SCREENING PROGRAMMES

In addition to the essential elements common to all programmes summa-

rized earlier, there are additional elements required for successful cervical cytology screening programmes:

### **Training of relevant health care professionals**

There are many types of health care professionals that will require training:

- The smear-takers (primary care practitioners or nurses). These individuals must be technically competent and capable of achieving good rapport with patients.
- The smear readers (up to the level of cytotechnologists).
- Cytopathologists.
- Histopathologists.
- Colposcopists.
- Gynaecologists.
- Programme managers.

Attention should be given to the assembly of the project team. All team members must be able to work well together. A dysfunctional team is unlikely to succeed.

Significant political issues usually must be resolved during team assembly.

The following team components should be clearly defined up front, with the understanding that changes may be made in team composition as the programme evolves:

- 1) Institution(s) and individuals who will be in charge of organized community outreach and Pap smear collection.
- 2) Individuals who will be in charge of contacting women with abnormal test results should be specifically designated.
- 3) Institution(s) and individuals who will be in charge of the centralized cytology laboratory;
  - a) Centralization facilitates quality control efforts, reduces overhead costs, assists in tracking the screened population, and catalyses introduction of improved technologies and procedures.
  - b) It is easier, politically, to centralize emerging laboratory services than to centralize pre-existing laboratory services.
- 4) Institution(s) and individuals to whom women with abnormal cytology test results will be referred.
- 5) Institution(s) and individuals who will be in charge of the centralized pathology laboratory to evaluate biopsy specimens (should be the same laboratory to which smears are sent for examination).
- 6) Institution(s) and individuals to whom women with invasive cancer will be referred.
- 7) Institution and individuals that will be in charge of quality control.

## **An agreed decision on the priority age group to be screened**

The priority age group to be screened should be defined by the age-related incidence of invasive cancer of the cervix in the country, not on the basis of the percentage distribution by age of clinically detected cases of cancer in the country. In most countries, it will be found that the majority of smears are being performed on young women, who are at low risk of presenting with invasive cancer within the next 5 years. Almost invariably it will be determined that the priority age group for initial screening are women age 35–54 years.

The most important screen is the first, and priority must be given to ensuring that as high a proportion of the target population as possible is screened once before attention is diverted to recalling women for subsequent screens.

## **Adequately taken and fixed smears and their preparation**

Inadequate collection of cellular material from the transformation zone and inadequate preparation, fixation and processing of the smear are major causes of false negative results (42-44).

An extended tip spatula (45), the combination of the Ayre spatula and endocervical brush and Cervex-brush spatulas (46,47) allow adequate collection of the target zone for preparation of conventional smears. The use of the Ayre spatula or cotton tip applicator alone should be avoided (45,48,49).

The speed of fixation is very important (the time between spread of material on the glass and fixation should be minimized to a few seconds). Fixation with alcohol has been shown in field circumstances to be adequate. Commercial fixative sprays are an alternative, but are more expensive.

Smear-takers need sufficient training. Several illustrated guidelines are available and are very useful tools. (50–52).

The laboratory should introduce a mechanism to monitor the proportion of inadequate smears submitted by the individual smear-takers. Those with >10% inadequate smears should undergo hands-on retraining in smear-taking.

## **Efficient and high quality laboratory services**

High quality laboratory services are essential to effective cytology screening. If it is possible to solve transport problems, the greater the centralization of such services the more efficient the laboratory will be. In small countries, this could imply a single central (national) laboratory. In large countries,

several regional laboratories will be required. In any case, a minimum throughput will be required to ensure adequate quality and efficiency. This minimum has been variously defined as 15–25,000 smears per annum (20, 26), or a work load justifying the employment of at least three technologists, who can each be expected to examine approximately 50 smears in an 8-hour day. The average time required for the interpretation of a one-slide gynaecological smear by an experienced cytotechnologist is estimated to be approximately 6 minutes (5 minutes for reading and 1 minute for reporting and handling) (53).

Small laboratories tend to use or interpret reporting systems inconsistently. Reports sent to clinics are not well understood by clinical staff, which may explain why so many women are requested to return for an unnecessary repeat smear. Quality control in laboratories also varies with some having outstanding systems and others having none at all. Computerization of laboratories is not standard and some laboratories use a paper-based system only. There is often no linking between cytology and histology reports making quality control a problem.

### **Quality control of cytology reading**

Quality control programmes must be introduced in all cytology laboratories. A 10% full re-screening of negative smears is ineffective and is not recommended. Rapid re-reading of 100% of negative slides is effective (54), but may be outside the reach of low resourced programmes. Careful evaluation of detection rates by the smear reader and special evaluation of those with rates out of line with expectation may help to identify poor performers. Continuous programmes of retraining are ideal. Participation of the laboratory in an external quality control programme, such as that organized by the Pan-American Health Organization (PAHO) for many Latin American countries (REDPAC (Panamerican Network of Cytology)) is one option for large laboratories.

### **A means to rapidly transport smears to the laboratory**

Delay in transportation of smears should be avoided as far as possible. Part of the budget for the programme should be dedicated to the cost of transporting specimens.

### **A mechanism to inform the screened women of the test results in an understandable form**

Women must be informed of the results of their test, in a form they can

understand. In particular, they will not understand (and may be misled and/or inappropriately alarmed/relieved) by a copy of the laboratory report. Indeed, the need for understandable reports also extends to communications with health care professionals at the primary health care level.

### **A mechanism to ensure that women with an abnormal test result attend for management and treatment**

In many programmes in middle-income countries, those with an abnormality commonly fail to return to the clinic for management. This is a general problem for all programmes that require referral for subsequent diagnosis and treatment (as distinct from a test and treat policy as discussed below for VIA). Practical see and treat policies for cytology have not yet been devised. The basic responsibility for ensuring such referral resides at the primary care level, rather than at the level of the individual woman. A special cadre of health visitors whose responsibility it is to ensure that women with abnormal test results do attend for diagnosis and management may have to be appointed. An evaluation of the cost-effectiveness of different screening policies in South Africa identified failure to attend for investigation and treatment of abnormalities as a major factor in reducing the cost-effectiveness of cytology (55).

### **Trained (licensed) colposcopists**

In most middle-income countries, colposcopists will either already be available, or can be trained. A licensing (training and certification) system is desirable, and careful ongoing evaluation of their performance should be an integral part of the programme.

### **An accepted definition of an abnormality**

There has been considerable confusion on the type of abnormality sought in cervical screening. The initial programmes in developed countries concentrated on detection of carcinoma *in situ*, later dysplasia was identified, but this was usually observed by repeat smears. Only recently have changes in classification systems resulted in action following what used to be labelled as atypical (or Class 2) smears, or borderline abnormalities, classed in the Bethesda system as atypical squamous cells of undetermined significance (ASCUS) (56). A new version of the Bethesda system has recently been devised, with two ASCUS-type designations: ASC-US (same as before) and ASC-H (cannot exclude high-grade squamous intraepithelial lesion (HSIL)) (57).

In the USA, the adoption of the Bethesda system dramatically increased

the proportion of smears with cytological abnormalities that appeared to require clinical attention. The new terminology increased the overall proportion of low-grade lesions by combining the original mild dysplasia category with cytological abnormalities consistent with koilocytotic atypias into the low-grade squamous intraepithelial lesion (LSIL) designation. Shortly after the adoption of the Bethesda classification it was estimated that ASCUS and LSIL comprised 9.4% of all Pap smears and up to 12.5% of smears in certain ethnic groups (58). As cytopathology laboratories gained experience with the classification, a decrease in the proportion of ASCUS (some 4-5%) and LSIL (some 2%) smears occurred (59).

There is currently agreement that cytology indicative of high-grade lesions (CIN II-III or moderate and severe dysplasia plus carcinoma *in situ* or HSIL in the Bethesda system) should engender immediate referral for colposcopy. Low-grade cytology (LSIL or ASCUS), under circumstances where women can be followed with regular cytology, should be so managed, and only referred for colposcopy if repeat smears at 6-month intervals show evidence of cytologic progression (26). As the large majority of low-grade lesions resolve spontaneously (37,60,61), there is no urgent need for treatment. The programme must provide specific guidelines on this issue.

### **A mechanism to follow-up treated women**

There are appreciable failures of treatment, so that follow-up (as a minimum with a repeat smear, but if possible with colposcopy), should be ensured. Those with high-grade abnormalities should be followed annually for at least 5 years before they are returned to routine screening.

### **Decision on frequency of subsequent screens**

The previous WHO recommendation was that when 80% of women aged 35–40 years have been screened once, screening frequency should increase to 10-yearly and then 5-yearly for women aged 30–60 years, as resources permit (22). To date, data are not available that suggest that these recommendations be revised. However, on the basis of modelling different approaches, it has been suggested that other intervals may be appropriate (such as five-yearly screening from the age of 35 for a total of three tests in a lifetime) (55). However, increasing the frequency of screening, and extending screening to younger age groups, does not compensate for deficiencies in laboratory quality or of population coverage.

There has been some criticism of the estimates made by the International Agency for Research on Cancer (IARC) Working Group on Cervical Cancer Screening (38), which are used to justify relatively infrequent screening



frequencies (22,23,26). Nevertheless, a re-evaluation of the models and computations of the effect of low coverage and reduced sensitivity, as might only be achievable in practice in some middle-income countries, still indicate that when resources are limited, the highest priority group for screening is those aged 35 or over, and that screening every 5 years will achieve a major impact (55,62). That this is not theoretical is proven by the success of the programme in Finland based on 5-yearly cytology screening for those age 35–59 (63).

### **A mechanism to invite women with negative smears for a subsequent smear**

Whatever the decision on the frequency of repeating screening (see above), it will be necessary to actively invite women to return for screening when their next smear is due. The appropriate mechanism will usually involve a similar mechanism to that used to invite women for their first smear.

## **ELEMENTS THAT INTERFERE WITH THE DEVELOPMENT OF SUCCESSFUL CERVICAL SCREENING PROGRAMMES**

The converse of the essential elements discussed above are the most critical in terms of failures of programmes. However, there are some elements that may require specific attention in some countries.

### **Over-reliance on reproductive health services for screening**

In most middle-income countries, screening will already be in place but may be largely restricted to the maternal and child health services (reproductive health). Unfortunately, women screened in such services tend to assume there is no need for screening after they have completed their attendance, and no mechanism is put in place for screening older women. A major role for the new or revitalized programme will be reallocating resources to ensure that the target group for the programme is screened.

### **Opportunistic screening**

Opportunistic (spontaneous) screening will often be ongoing. There is evidence that such screening is far less efficient and effective than organized programmes while costing more, because those screened tend to be at low risk for the disease (25). Reallocation of these resources to the organized programme will often save money.

## Low coverage of the target group with screening

Throughout this report, low coverage has been identified as the most critical reason for failure of cervical screening. Evidence presented at the consultation from the National Programme in Costa Rica shows that the previous reports that high screening coverage in that country produced no reduction in incidence of the disease, were flawed. These reports were based on a faulty process of estimating coverage, such that smears were counted rather than individuals entering the programme, and they did not account for the fact that many women had repeat smears. Coverage must, therefore, be measured on the basis of individuals recruited into the programme, rather than smears performed.

## Setting too low a threshold for referral for colposcopy, i.e. over-treating non-progressive disease

As the large majority of low-grade lesions regress (37,60,61), a policy based on referral for colposcopy of cytology reported as low-grade (or ASCUS) will increase costs, yet have minimal impact on the disease. The programme should be capable of tracing and following women who are screened. Thus it should be possible to follow these women by repeat cytology every 6 months until it is possible to determine whether there is cytological evidence of disease progression.

## THE STRENGTHS OF THE CONVENTIONAL CERVICAL CYTOLOGY TEST

Cervical cytology is known to reduce cervical cancer incidence and mortality, particularly in organized programmes, though in North America and some countries in Europe benefit was obtained with excessive opportunistic screening. In addition, the test has the following strengths:

- Decades of experience in its use.
- High specificity.
- Lesions identified are easy to treat.
- Relatively low cost.
- Qualified manpower and laboratory resources exist in most countries.

However, there are limitations of the test. These include:

- The test is embarrassing and is difficult to comprehend in many cultures.
- Requires trained personnel.
- Smear adequacy not intrinsically obvious.

- It is necessary to recall women for further tests if the smear is inadequate, or for evaluation if an abnormality is suspected.
- In most laboratories only moderate sensitivity is achieved and reproducibility is poor.
- Cytology is unable to distinguish progressive disease from that destined to regress. This is true for both reported low-grade and high-grade lesions, with the probability of progression being much lower for low-grade abnormalities (37,60,61).
- Cytology may be less effective in older women. This reflects reduced exfoliation of lesions in such women, and the fact that the transformation zone tends to move to the endocervical canal. However, if women have been adequately screened over the age range 35–54 and have never had an abnormal smear, they are at low risk of disease and screening can stop.
- Cervical screening using cytology requires considerable management effort and coordination because of the number of different agencies involved. This can prove expensive.
- It is impossible to abolish the disease by screening. There will always be cases of invasive cancer that occur despite screening because the biology of the disease in that individual resulted in a progression that was too rapid for timely detection to result in effective treatment. In addition, no programme can guarantee 100% coverage or total effectiveness of the screening process. Thus programmes are likely to reach an irreducible minimum of invasive cancer in the population served, that will probably be of the level 10–20% of the incidence in the absence of screening. It is important that this is understood by participants in the programme, and by those who fund and support the programme.

## THE IMPLICATIONS OF DIFFERENT METHODS OF SAMPLING

Liquid-based cytology is currently too expensive for most developing countries. In addition, it is important to recognize that liquid-based cytology does not compensate for an inadequate smear being taken. Thus at present, liquid-based cytology needs to be further evaluated as to its viability in developing countries.

However, experience in its use in developed countries suggests it has a number of advantages over conventional cytology, and in some circumstances these advantages could result in an overall cost-effectiveness of the process being improved, even though the test itself is more expensive. The advantages of liquid-based cytology are that it:

- Produces a uniform layer of cells, representative of those present in the smear.
- Has the potential to:
  - Improve sensitivity.
  - Improve specificity.
  - Increase throughput in the laboratory.

However, liquid-based cytology has a number of additional requirements, or will require readjustment of existing programmes. These include:

- Retraining of smear-takers.
- Transport and storage of vials by different approaches.
- Retraining of cytotechnologists.
- Purchase of new equipment.
- Review of quality assurance schemes as the targets change (the expected numbers and ranges will change).

Nevertheless, experience in the UK and USA suggests that the learning curve is brief, and that technologists who are trained for the first time on liquid-based cytology perform better, and more rapidly reach an adequate level of expertise, than those previously trained to examine conventional smears. For those taking smears, a course of 2–3 days duration is sufficient.

## THE IMPLICATIONS OF DIFFERENT METHODS OF SMEAR READING

- Smears can be read by specially trained lay workers.
- Smear reading is a good occupation for some disabled workers.
- Computer-assisted reading shows promise in improving sensitivity and possibly specificity, but the cost is too high for most developing countries at present. Furthermore, in such countries, maintenance of automated readers will require attention.

## THE CIRCUMSTANCES WHEN CERVICAL SCREENING PROGRAMMES SHOULD BE RECOMMENDED

- Cervical cancer control is judged to be a sufficiently high priority as part of a National Cancer Control Programme.
- The essential elements discussed earlier are in place, or can be provided, and it is intended that they be sustained in the country.
- The political and professional will for the relevant changes has been secured.

However, it is rarely possible to initiate a nationwide programme *de novo*, since until screening is organized in at least one area, the particular barriers that may be specific to that country will not be identified. Therefore, a pilot (demonstration) project should initially be put in place in one or more areas in the country, and these should be successfully completed (in terms of the process and impact measures previously defined) before the programme is extended to the whole country.

## RESEARCH ISSUES ON CERVICAL SCREENING PROGRAMMES IN DEVELOPING COUNTRIES

- Determine culturally relevant ways to secure an educated target population.
- Determine a cost-effective approach to securing compliance with screening in each country.
- Evaluate different approaches to manage programmes and improve their performance.
- Determine whether a vertical (top down) or horizontal organization is optimal for the country.
- Determine optimal ages for initiating and stopping screening.
- Determine the required period of intensive follow-up for those treated for high-grade lesions.

## RESEARCH ISSUES ON CERVICAL CYTOLOGY IN DEVELOPING COUNTRIES

- Conduct a detailed evaluation of smear-taking devices.
- Longitudinally determine the sensitivity of smear testing in developing countries.
- Evaluate the need for repeat smears after the first smear – by age.
- Evaluate the merits of liquid-based vs. conventional cytology with study designs that permit measurement of sensitivity and specificity.
- Develop laboratory quality control strategies that are relevant to the needs of the country.
- Develop a cytology classification system that is more relevant to the natural history of the disease, and the needs for a system that concentrates management resources on the CIN III component of high-grade lesions.

## VISUAL INSPECTION WITH ACETIC ACID APPLICATION (VIA) AS AN ALTERNATIVE APPROACH TO CYTOLOGY SCREENING IN LOW-INCOME COUNTRIES

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# 4

Even though cytology screening may be feasible in middle-income countries, there are technical, human resource and financial constraints in implementing such programmes in low-income countries. In view of this, alternative methods based on visual examination of the cervix have been investigated for the control of cervical cancer in low-resource settings (64-67). The visual methods of screening include unaided visual inspection of the cervix ('downstaging'), visual inspection with 3-5% acetic acid (VIA) (synonyms: direct visual inspection (DVI), cervicoscopy, aided visual inspection), VIA with low-level magnification (VIAM), cervicography, and visual inspection with Lugol's iodine (VILI). Downstaging has been shown to be inaccurate in detecting disease, particularly cervical pre-cancers (64), and is not further considered in this report.

Among the visual inspection approaches, VIA has been more widely investigated for its performance characteristics (accuracy) in detecting cervical neoplasia, in various settings, by different providers. VIA involves naked eye examination of the 3-5% acetic acid-swabbed uterine cervix without any magnification, usually by nurses and other paramedical health workers, with illumination provided by a bright light source, such as a halogen lamp. A positive test is the detection of well-defined, dull acetowhite lesions on the cervix. The objective of VIA is to detect acetowhite lesions leading to the early diagnosis of high-grade cervical intraepithelial neoplasia and early pre-clinical, asymptomatic invasive cancer. A major advantage with VIA is that it is a real-time screening test, as the outcome is known immediately after the administration of the test, so that further investigations/treatment can be planned and carried out during the same visit.

Historically, before the advent of Pap smears and routine cytology-based screening programmes, health care providers relied on inspection of the cervix to detect abnormalities. After the 1950s, when cytology smears became the standard for cervical screening, the colposcope (initially developed in the 1930s) began to be used increasingly to further investigate screen-positive women and to direct biopsies in order to confirm screening findings. Eventually, VIA was explored as an adjunct to the Pap smear to decrease the false negative rate of cytology and for more efficient identification of women for colposcopic triage. These studies, and the need for a suitable alternative for cervical cytology, led to the investigation of the

accuracy and efficacy of VIA as a primary cervical screening tool. Moreover, they have provided valuable insights into the test characteristics of VIA in detecting cervical neoplasia. The results indicate that VIA is at least as sensitive as conventional cytology in detecting high-grade lesions, but that its specificity is lower. Thus, VIA appears to be the most promising low-technology alternative to cytology (26). VIA is currently being investigated for its efficacy in reducing incidence of and mortality from cervical cancer.

## TEST CHARACTERISTICS AND CURRENT LEVEL OF EVIDENCE FOR VIA AS AN ALTERNATIVE SCREENING APPROACH

The basic step in assessing the utility of a screening test is the determination of its test characteristics in terms of sensitivity, specificity and predictive values. Consistently low sensitivity and specificity of a given test preclude its further evaluation for reducing incidence and/or mortality from a given disease. A summary of key cross-sectional studies addressing the test characteristics of VIA is presented in Table 1. Ottaviano and La Torre (68) examined 2400 women using VIA and the colposcope. VIA detected abnormalities in 98.4% of patients assessed colposcopically as having an abnormal transformation zone and it correctly identified 98.9% of normal cases. In a study involving 145 women attending health clinics, the reported odds ratio for a positive cytology was 6.6 if the VIA test was also positive (69). In a study among 2827 women, Slawson et al (70) demonstrated that VIA might be helpful in reducing referrals for colposcopy. Van Le et al (71) found that VIA resulted in an additional 15% of CIN cases being identified among cytology-negative women, but 40% of women with positive VIA underwent unnecessary colposcopy (false positives). Frisch et al. (72) found that combining a negative cytology and negative VIA test resulted in a negative predictive value (NPV) of 91% – greater than that obtained for cytology alone, but with some loss in positive predictive value (PPV). These studies demonstrated the potential value of VIA as a viable screening approach, but did not establish its test qualities as a primary screening method.

Cecchini et al (73) provided evidence on the accuracy of VIA. VIA was more sensitive than cytology, but less specific. Additionally, screening sequentially using VIA was more cost-effective than with cervicography.

Subsequently, six published studies on VIA as a primary screening modality have been carried out in developing countries. In the study by Megevand et al. (74) in South Africa, VIA and cytology were performed in a mobile unit equipped to process smears on site. In that setting, VIA

*Table 1. Summary of key VIA study results*

Reference	N	Purpose <sup>1</sup>	Key statistics	Key results
Ottaviano and La Torre (68)	2400	Compare VIA w/ colposcopy	True positive rate	98 % of abnormal TZ on colposcopy identified by VIA
Fiscor et al. (69)	145	Correlate cytology and VIA	Odds ratio (OR)	OR for cytology + & VIA+ was 6.6
Slawson et al. (70)	2827	Increase in CIN identified using VIA as adjunct to cytology	True positive rate/ % increase in CIN detection	VIA increased CIN detection by 30% / VIA alone detected 64% of CIN vs 68% for cytology alone
Denny et al. (75)	2944	Compare performance of 4 tests in detecting HGSIL+	Sensitivity ratio/ approximate sensitivity/ approximate <sup>2</sup> specificity	Sensitivity ratio to cytology = 0.85, p=0.16; estimated sensitivity = 67% for VIA vs 78% for cytology; estimated specificity = 83% vs 94%; PPV 11% vs 27%; NPV 99% vs 99%.
Van Le et al. (71)	85	Increase in detection rate of CIN with VIA	% increase in CIN detection	VIA detected 15% more CIN than cytology alone
Frisch et al. (72)	95	Evaluate VIA as a supplement to cytology	Positive predictive value (PPV)/ Negative predictive value (NPV)	NPV of VIA and cytology together was 91% – significantly more than for cytology alone (= 67%); PPV of both was 57% vs 82% for cytology alone
Ceccini et al. (72)	2105	Diagnostic accuracy of VIA	Sensitivity/ specificity	Sensitivity of VIA = 88% vs 63% for cytology; specificity = 75% vs 99% for cytology; PPV 1.3% for VIA vs 24% for cytology
Megevand et al. (74)	2426	Value of VIA as an alternative to cytology	PPV	Sensitivity of VIA for HGSIL 65% vs 100% for cytology; specificity 97% vs 89%; PPV of VIA=26 % vs 10% for cytology
Londhe et al. (76)	372	Test qualities of VIA	Sensitivity/ specificity	Sensitivity of VIA= 72 % vs 13 % for cytology; specificity of VIA = 54% vs 96% for cytology
Sankaranarayanan et al. (77)	3000	Compare performance of VIA and cytology	Sensitivity ratio/ approximate <sup>2</sup> specificity/ McNemars	Sensitivity ratio = 1.05 p = 0.25 (Sensitivity estimate = 90 % vs 86 % for cytology) approximate specificity of VIA=92 %; approximate specificity of cytology: 91% PPV 17% for VIA vs 22% for cytology; NPV 99% vs 99%.
Sankaranarayanan et al. (78)	1351	Compare performance of VIA and cytology	Sensitivity ratio/ approximate <sup>2</sup> specificity/ McNemars	Sensitivity ratio = 1.54, p<0.001 (Sensitivity estimate = 96% vs 62% for cytology) approximate specificity of VIA = 68%; approximate specificity of cytology: 90%; PPV 15% for VIA vs 25% for cytology; NPV 99% vs 97%.
University of Zimbabwe/ JHPIEGO (79)	10934	Test qualities of VIA	Sensitivity/specificity	Sensitivity = 77% for VIA vs 44% for cytology/specificity = 64% for VIA vs 91% for cytology; PPV 19% vs 33%; NPV 96% vs 94%

TZ: Transition Zone  
NPV: negative predictive value  
PPV: positive predictive value

1 Main study purpose stated in the article  
2 Approximate because of problems with verification bias



detected 65% of high-grade squamous intraepithelial lesions (HSIL) confirmed by the reference standard. In another study from South Africa, Denny et al. (75) looked at the comparative performance of VIA, cytology and three other tests including human papillomavirus (HPV) testing, all performed in a primary health care clinic. VIA and HPV testing were similar to cytology in their ability to detect HSIL+. VIA, however, yielded more false positives. Three Indian studies in the late 1990s provided additional evidence on the performance of VIA as an alternative to cytology as a primary screening test. Londhe et al. (76) studied 372 women who underwent VIA, cytology and colposcopy in a gynecology outpatients clinic. VIA identified 78% of HSIL (and 1 cancer) diagnosed through colposcopy – 3.5 times more than those identified via cytology. Sankaranarayanan et al. (77) studied 3000 women, who had VIA and cytology provided by trained cytotechnicians. The performance of both tests was similar (sensitivity ratio of 1.05) in detecting moderate/severe dysplasia. In the third Indian study (78), nurses were trained to provide VIA and all recruits were subjected to both VIA and conventional cytology. VIA detected considerably ( $P < 0.001$ ) more moderate/severe lesions than cytology, but was significantly less specific. In these studies, the reference investigation by colposcopy was carried out only in test-positive women and a small proportion of test-negative women, with the result that these studies suffered from verification bias (36). It is quite likely that sensitivity may have been over-estimated as a result of verification bias, although the extent of this bias is difficult to assess because it is a function of the true prevalence of disease in each setting.

A study from Zimbabwe comparing VIA and cytology performed by nurses in primary health clinics was the first to yield direct estimates of sensitivity/specificity, because all women testing negative *or* positive on screening were offered the reference standard, thus avoiding verification bias (79). In that study, the sensitivity of VIA (for HSIL +) was 1.75 times higher than cytology (76.7% versus 44.3%, respectively), whereas the specificity was 1.4 times lower (64.1% versus 90.6%).

The range of estimated VIA sensitivity from the seven cross-sectional studies that specifically addressed the accuracy of VIA (72–78) was 66% to 96% (median 84%). For specificity, the range was 64% to 98% (median 82%). The positive predictive value ranged from 10–20% and the negative predictive value from 92–97%. The weighted mean sensitivity and specificity of VIA from these studies were 81% and 83%, respectively. Of interest, the above ranges are considerably narrower than those observed from cross-sectional cytology studies (i.e., 20–85%) over the past few decades (32,33). In studies where VIA was compared to cytology in the same setting, VIA performed similarly to (sometimes better than) cytology in terms of detecting high-grade lesions

or cancer, but was less specific (73–79). The addition of magnification to VIA (VIAM) does not seem to improve the accuracy of the test (75).

A useful complement to this qualitative review of the evidence on VIA to date would be a meta-analysis aimed at providing a quantitative summary measure of test performance indicators of VIA. One such analysis involving three VIA studies conducted before 1996 compared the ability of VIA, cytology and a number of other tests to identify any precancerous lesions (versus HSIL+) (78). VIA had a substantially higher area under the receiver operating curve (0.85) compared to cytology (0.70) in this study. Given that VIA test performance indicators (especially sensitivity) from more recent studies are generally higher than those from the three studies used in that analysis, future meta-analyses will likely provide even more convincing evidence on the test characteristics of VIA. Thus, studies to date support the conclusion that VIA performs similarly, if not better than, cytology in the detection of high-grade cervical cancer precursors.

Notably, however, all these studies, except two, suffered from verification bias (36). In addition, different definitions of a positive VIA test were used in the various studies, making comparison of test performance in the studies more difficult. Furthermore, performance characteristics of VIA have to date been studied in cross-sectional studies involving prevalent cases and information based on longitudinal studies is not yet available. Some of the ongoing follow-up and randomized intervention studies in different settings will provide this information in the near future.

The current level of evidence available for VIA as a screening test for cervical cancer and its precursors suggests that the test can be used for early detection to investigate symptomatic and high-risk women. Evidence concerning the ultimate effectiveness of a screening programme based on VIA to reduce incidence of and mortality from cervical cancer is not yet available, though efforts are underway to generate such information. However, as already emphasized, incidence and mortality in a cervical screening programme are affected by many factors including access to screening, participation, compliance to investigations, treatment and follow-up and quality of treatment, and do not only depend on the accuracy of screening.

Information on test characteristics alone does not suffice for making decisions regarding whether VIA may be adapted for use in population-based organized screening programmes in routine public health practice. Evidence on its comparative efficacy and cost-effectiveness versus cytology screening in reducing incidence and/or mortality, as well as information on short-term and long-term consequences arising from decisions taken based on screening test outcomes, is essential for recommendations leading to public health policies. Such evidence may ideally be generated from randomized controlled trials.

Currently, there are three large randomized trials ongoing in India (Dindigul district, Tamil Nadu; Mumbai (Bombay) city and Osmanabad district, Maharashtra) that are addressing the efficacy of VIA screening in reducing the incidence of and mortality from cervical cancer. The programme in Osmanabad district addresses the comparative efficacy of conventional cytology, VIA and HPV testing. The programmes in Dindigul district and Mumbai address the efficacy of VIA in reducing the incidence of cervical cancer. A three-arm, prospective randomized intervention trial in South Africa is currently addressing the comparative safety, acceptability and efficacy of screening women with VIA and HPV DNA testing. This trial is also investigating the effects of immediately treating women who screen positive with cryotherapy, performed by nurses in a primary health care setting. Outcome measures include reduction of high-grade cervical cancer precursors in treated versus untreated women, followed over a 12-month period. A large demonstration project of VIA, which is ongoing in the San Martin region of Peru, aims to investigate the effectiveness and acceptability of VIA integrated with the health services. Those screening positive by VIA are referred for magnified visual inspection after application of acetic acid (VIAM) and immediate biopsy and cryotherapy are provided if indicated by VIAM. A similar intervention involving VIA and VIAM has been initiated in Western Kenya. These studies are likely to provide the required evidence on the longitudinally-derived sensitivity and the cost-effectiveness of VIA in decreasing cervical cancer incidence and mortality.

A computer simulation cost-effectiveness (CE) analysis for Thailand comparing VIA, HPV and cytology-based screening and treatment algorithms suggests that VIA-based approaches are the most cost-effective under the conditions characterizing many developing countries (80). Furthermore, in a study simulating conditions in South Africa, VIA followed by immediate cryotherapy was associated with a projected 26% reduction in incidence and was found to be economical in comparison to no screening (55). Additional CE analyses (those performed to date have been based on mathematical modelling) and the results of ongoing prospective trials will validate these findings.

## DEFINITION AND REPORTING OF TEST OUTCOMES

The most common form of reporting the VIA test outcome involves negative and positive categories (75,77,78), depending upon the absence or presence of acetowhite lesions and clinical signs of invasive cancer, although an indeterminate or inconclusive category is being used by some investigators. A positive test is generally based on the detection of well-defined,

densely opaque acetowhite lesions in the transformation zone closer to the squamocolumnar junction, one minute after the application of 3-5% acetic acid. The criteria for a negative test have included one or more of the following: no acetowhite lesions, faint ill-defined translucent acetowhite lesions, endocervical polyps, nabothian cysts, dot-like acetowhite lesions, acetowhite lesions far away from the transformation zone and a prominent squamocolumnar junction. However, these categories and criteria (to define the categories) vary to some extent among different study groups. The colour intensity (pale or dull white) and location (in, or close to, far away from the squamocolumnar junction/transformation zone) of the acetowhite lesions has been a major determinant in deciding the outcome of the test in some of the reported and ongoing studies (78). There have been attempts to grade the severity of a positive outcome as ‘faintly’ or ‘strongly’ positive depending on the intensity of whiteness. However, such categorization had very little impact on test accuracy. The test-positive rate varies from 10–35% in most reported and ongoing studies. This variation is not surprising, since VIA is an entirely provider-dependent screening test. The test positivity rate has been approximately 10–15% in reported and ongoing studies categorizing dull acetowhite lesions with well-defined margins (78) located in the transformation zone as a positive test, while a higher test positivity rate (18–35%) (75,77,79) has been observed in studies categorizing any acetowhite lesion as a positive outcome.

One of the reporting schemes used categories such as ‘Normal’, ‘Atypical’, ‘Abnormal’ and ‘Cervical cancer’, in which the abnormal category corresponds to the positive category in other reporting schemes (79). An ‘indeterminate’ category has also been used when no distinct acetowhite lesions or somewhat doubtful lesions are visible, or when the cervix cannot be adequately assessed. The different definitions and schemes might have contributed to some misclassification, observer variation, different thresholds for referral and some difficulties in comparing the results across the studies. The necessity of uniformly reproducible categories for reporting the results of VIA has been increasingly appreciated. A consensus is currently emerging that the visualization of dense acetowhite lesions with sharp borders located in the transformation zone, close to the squamocolumnar junction, constitutes a positive VIA test outcome.

## DIAGNOSTIC EVALUATION AND MANAGEMENT OF LESIONS DETECTED BY VIA

VIA is a real-time screening test as the result is reported immediately after the screening examination. This allows planning further investigation and

treatment in the same session as the screening examination, providing potential logistic advantages in linking investigation and treatment at one visit. In contrast, recall of women for diagnostic investigation and treatment is necessary for cervical cytology.

The investigation and follow-up algorithms for women with a positive VIA test have, to date, generally involved colposcopy and biopsy. If abnormal cervical tissue is identified during colposcopy, the region with the greatest abnormality is biopsied using a sharp punch biopsy forceps. Single or multiple biopsy specimens may be obtained. Treatment is then provided by excision or destruction (ablation) of the abnormal cervical tissue, after results of the biopsy are available. However, in various studies, other options of management have been offered to VIA positive women. These include:

- Referral for colposcopy with sampling for histology and treatment given on the basis of the colposcopic diagnosis (with retrospective access to histological diagnosis).
- Referral for magnified visual inspection (VIAM) with histology sampling and immediate treatment with cryotherapy.
- Referral for colposcopy and treatment on the basis of colposcopic diagnosis (without histological diagnosis).
- Referral for immediate treatment with cryotherapy on the basis of a positive VIA test (without colposcopic interface and histological diagnosis) ('test and treat' or single visit approach).

All of these approaches to screening and treatment, based on a positive VIA, are still being evaluated in terms of safety, acceptability to women, feasibility and effectiveness in eradicating pre-invasive cervical disease. It is clear that a screening programme based on VIA requires resources and technologies for investigation, diagnosis, treatment and follow-up of screen-positive women. Paradoxically, these systems are not readily available in many high-risk countries of sub-Saharan Africa or in Latin America and South and South-East Asia. This is why some investigators are currently evaluating the safety and acceptability of the 'test and treat' approach after clinically excluding invasive cervical cancer, without colposcopy/biopsy diagnostic interfaces in the overall management, as an option of disease control in the lowest of the low-resource settings (81). However, it is clear that screening and immediate treatment based on VIA would invariably result in some over-diagnosis and over-treatment, as the test suffers from low specificity. The long-term effects of such over-treatment carried out in field conditions remain to be established. Also, it is not clear whether the resources and infrastructure required for a 'test and treat' approach are substantially lower than those for the other management approaches. One of the options that has not yet been explored

is no immediate treatment with VIA repeated after a period of time, as has been done traditionally with cervical cytology screening.

Ablative procedures, such as cryotherapy, may be used to treat lesions located on the ectocervix that occupy less than three-quarters of the transformation zone and satisfy the following criteria:

- lesions do not extend into endocervical canal;
- the squamocolumnar junction is fully visible;
- the lesion can be entirely covered by the largest probe (25 mm);
- invasive cancer has been excluded.

It is recommended that lesions not satisfying these criteria are treated by loop electrosurgical excision (LEEP) or cold knife/laser conization. Regardless of modality, healing takes 3–4 weeks. During this period, abstinence from sexual intercourse is recommended to prevent the possibility of infection and to promote healing. There are insufficient data on the impact of sexual intercourse during this healing period.

## TRAINING ISSUES

Comprehensive competency-based training is absolutely essential to provide reliable screening examinations with VIA. The objective of VIA is to recognize acetowhite lesions harbouring high-grade CIN II-III and early preclinical asymptomatic invasive cancer. An efficient training course, preferably given over a short period (1–2 weeks), supported by a teaching manual, is essential for health workers to provide quality VIA. Training screeners to perform VIA has not been standardized across various studies. Training in the administration and reporting of VIA has been carried out in sessions lasting for 3 days to 2 weeks in most of the reported study settings. A learning period has been recognized following the training sessions. In studies conducted to date, the proportion of positive screens identified by newly trained screeners has ranged from 25–35%, which later decreased to 10–18% in most instances. Training manuals have been developed and are in widespread use in the context of ongoing studies (81,82). Written manuals, charts, atlases and hands-on clinical training have been used to train the screeners.

The important components of a VIA training course can be summarized as follows:

- Anatomy of the female genital tract.
- Physiology: normal secretions, development of transformation zone, squamous metaplasia.
- Pathology: Infection and inflammation, causes of cervical carcinogenesis and its natural history.

- Clinical: techniques of per speculum examination, digital per vaginal examination, bimanual palpation, per rectal examination; recognition of normal anatomical components; recognition of clinical signs of metaplasia, polyps, leucoplakia; signs of infection and inflammation; recognizing and scoring acetowhite changes; gross appearance of invasive cancer.
- Assessment of provider skills and reorientation.

A variety of personnel such as doctors, nurses, health workers, auxiliary nurse midwives, paramedical technicians, and school graduates may be trained in VIA. The training must involve tutorials in the anatomy, physiology and pathology of the female genital tract, as a lack of such knowledge may affect the way the provider administers and interprets the test. The test providers' results of VIA should be correlated with those of colposcopy and, if possible, histology. Both external monitoring and self-assessment of providers by documenting test-positive and false-positive rates provide objective parameters to assess the skills of the provider and the outcome of training.

Useful benchmarks are the proportion of women examined who scored as acetopositive and the proportion of acetopositive women ultimately diagnosed with dysplasia. With sufficient skills, 10–20% of women examined may be scored as acetopositive and one or more of 5 VIA-positive outcomes result in the diagnosis of dysplasia or cervical intraepithelial neoplasia of any grade.

There is a learning curve in acquiring the skills of providing VIA. The provision of VIA improves depending on (i) the innate interest of the provider, and (ii) the provider's experience. Close monitoring and retraining may be required on an individual basis to maintain sufficient skills of screeners.

## ADVANTAGES AND LIMITATIONS OF VIA

The advantages and limitations of VIA as a screening test are presented in Table 2.

Many aspects of VIA make it an attractive test for use in low-resource settings. It is a simple, inexpensive, low-technology test that requires minimal infrastructure for use. Its cross-sectional sensitivity appears to be similar to cytology in detecting high-grade disease. It is possible to train workers on how to use this screening method in a short period of time (1–2 weeks). It is a real-time test in the sense that the results are available immediately, making it possible to institute further diagnostic investigations for test positive women, as well as plan and offer treatment during the same visit. The test appears to be comparable in reproducibility to other tests (83). A VIA-

based screening programme may be readily integrated in the primary care level of health services.

However, the low specificity of VIA may result in over-investigation and possible over-treatment in test and treat conditions. The test positivity (referral) rate varies from 10–35% in most reported and ongoing studies. Adequate training of health workers is important to reduce false-positive referrals. To date, no standard quality control procedures are available for VIA. The test essentially identifies disease in the ectocervix only when the transformation zone remains on the visually exposed part of the cervix. Since the transformation zone recedes to the endocervical canal in postmenopausal women and the test has inherent difficulties in identifying endocervical disease, VIA may be of limited use in older women. How well VIA works in an integrated service delivery model, with other competing demands for provider time, knowledge and skills, is not yet proven – most of the data are from research settings in which dedicated providers largely perform VIA only. Performance decay over time may be an important problem to tackle.

## RESEARCH ISSUES

- Further research is essential in addressing methods for improving specificity, quality control, tests to be used to follow-up women who have been

Table 2. Advantages and limitations of VIA

<ul style="list-style-type: none"><li>• Test characteristics of VIA have been consistent across studies with different designs.</li><li>• Sensitivity of VIA in detecting high grade disease seems to be as good as cytology.</li><li>• VIA screeners can be trained more rapidly than cytotechnologists or medical technologists (3–14 day training courses have been reported).</li><li>• Real-time screening test: results are available immediately after the test</li><li>• VIA has comparable reproducibility to that of other tests.</li><li>• It is simpler and cheaper than other screening tests (e.g. cytology and HPV DNA testing)</li><li>• Can be performed in extremely low-resource settings.</li><li>• If treatment linked to screening were to be shown to be safe and effective, recall of women may not be necessary, thereby potentially minimizing default rates for treatment of women with positive screening tests.</li></ul>	<ul style="list-style-type: none"><li>• Low specificity compared with cytology (high rates of false positivity).</li><li>• High test-positive rate (10–35%).</li><li>• Low positive predictive value of a positive test (10–30%).</li><li>• Over-investigation and over-treatment of screen-positive women in test and treat conditions.</li><li>• No standardized methods of quality control.</li><li>• Training methods and competency evaluation not yet standardized.</li><li>• Essentially leads to detection of ectocervical disease.</li><li>• Uncertain how VIA works in an integrated service delivery model.</li><li>• Performance decay may be an important problem to tackle.</li></ul>
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treated, and competency and evaluation of skills of screeners and other health personnel involved in screening programmes. It remains to be determined whether sequential application of Lugol's iodine may reduce false-positive referrals from VIA.

- Uniform categories and criteria should be developed to report the results of VIA to facilitate valid comparisons of results across studies, as the results of VIA have been reported using different schemes, and varying criteria have been used to define the outcome. VIA is an entirely provider-dependent screening test. Clear standards to identify the abnormal lesions are essential to ensure that providers make appropriate judgement. Research should be directed to facilitating the development of a simple scoring system to objectively report the results of VIA .
- Screening with VIA invariably results in over-investigation and over-treatment in test and immediate treatment schemes. The long-term complications and safety of over-treatment in the context of VIA screening programmes needs to be established.
- The efficacy and cost-effectiveness of a screening programme based on VIA in reducing the incidence of and mortality from cervical cancer are not known and remain to be investigated. The currently available information on VIA is limited to data on accuracy, based on the detection of prevalent disease in cross-sectional study designs with no longitudinal follow-up. Satisfactory accuracy and reproducibility of a screening test in cross-sectional studies cannot be interpreted as evidence for potential effectiveness in reducing disease burden in VIA-based screening programme. The ongoing intervention studies in India, Peru, South Africa and other countries may provide valuable information on its longitudinal (programme) sensitivity, programme effectiveness in detecting high-grade lesions and preventing cervical cancer, as well as on safety issues.

## CONCLUSION ON VIA

The test performance of VIA suggests that it has similar sensitivity to that of cervical cytology in detecting CIN, but has lower specificity. Further research is required to improve its specificity without compromising sensitivity. Information from ongoing studies regarding its longitudinally-derived sensitivity, efficacy in reducing incidence/mortality from cervical cancer, its cost-effectiveness and safety will be useful in formulating public health policies to guide the organization of VIA-based mass population-based screening programmes in developing countries.

A VIA-based screening programme requires a large infrastructure for investigation, treatment and follow-up of the positive screens. It is not

known whether cost savings with a cheap test like VIA might be offset by the referral and investigation of a higher proportion of women. Since a programme based on VIA involves a certain level of over-treatment, the efficacy, safety and long-term consequences of such a programme also remain to be fully addressed. Thus, information from ongoing studies on these issues will be crucial in judging how appropriate and feasible it will be to introduce VIA-based cervical cancer screening programmes on a population-wide basis in low-income countries.

*VIA as an  
Alternative  
Approach  
to Cytology  
Screening in  
Low-Income  
Countries*



# HPV TESTS IN CERVICAL SCREENING PROGRAMMES; POSSIBLE ROLE IN MIDDLE-INCOME COUNTRIES

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# 5

## INTRODUCTION

The recognition that the vast majority of cervical cancer cases worldwide are caused by a restricted number of viruses, of the Human Papillomavirus (HPV) family (84), has led to novel opportunities for screening based on the use of tests for HPV exposure. These opportunities are now in an advanced stage of evaluation, largely due to the availability of standardized, Food and Drug Administration (FDA) approved, testing systems to detect the presence of HPV DNA in exfoliated cervical cells.

In terms of public health and also for practical purposes, all cervical cancer cases should be considered to be caused by HPV infection. Although some relevant cofactors are likely to play a role in the acquisition of HPV infections and their promotion to neoplastic growth, specific preventive practices targeting putative HPV-unrelated cervical cancers are, at present, unjustified.

As described in the first section of this report, screening programmes are complex public health strategies of which the screening test being used is only one component. As a consequence, the advances derived from the use of better tests would only result in noticeable reductions in mortality from cervical cancer if the screening programme in its entirety is functional and efficient over extended periods of time.

Many studies that have evaluated novel testing systems for screening using HPV DNA tests have been conducted in high-income countries where cytology is widely used. In many of these developed countries, current screening activities target different objectives in relation to cervical cancer. For example, reduction of costs by increasing screening intervals without reducing preventive efficacy.

The results of studies conducted in developed countries are thus only indicative of an expected result if applied to middle-income countries. The use of HPV tests in countries with low or very low health resources will not be considered in this report. Country-specific evaluations are required and no general rules can be firmly established at this point.

## RATIONALE FOR USING HPV TESTING IN CERVICAL SCREENING

In countries where cytology-based screening programmes are well developed, better screening tests may result in more efficient programmes, reduction of costs and reduction of the number of required screening events per woman. In countries where *de novo* screening activities are under consideration, the introduction of better tests and testing strategies has the potential to overcome the test limitations encountered by cytology-based programmes. This may accelerate the achievement of efficient systems.

The rationale for HPV testing in cervical screening programmes can be summarized as follows:

1. The strong association between HPV DNA in cervical cells, cervical intraepithelial neoplasia and cervical cancer. The association has been widely accepted as causal in nature. Some evaluations qualified the association as a necessary (absence of disease in the absence of HPV exposure) but non-sufficient (existence of exposure in the absence of disease) cause.
2. The high HPV-attributable fraction. The most frequently reported proportion of cervical cancer attributable to HPV ranges from 90 to 99 %. In terms of public health, this proportion indicates that the existence of HPV-negative cervical cancer cases is negligible and argues against devising any intervention targeting some putative HPV-negative cervical cancer cases.
3. The long time interval (> 15 years in some studies) between HPV infection and cervical neoplasia at the level of HSIL or more advanced lesions. The risk of cervical cancer is specifically related to persistent HPV infections and these can be recognized years before clinical symptoms.
4. The minimally invasive nature of the sampling procedure to perform an HPV test. From the patient's viewpoint, sampling for HPV DNA testing is identical to the procedures required for a standard cytology test. From the service provider's perspective, specimen sampling for HPV testing requires minimal additional training.
5. The availability of one standardized, FDA approved, assay to detect HPV DNA (current hybrid capture technology). This assay has been shown to be consistent and highly reproducible. Standardized equipment is transferable to the clinical setting and inter-laboratory and intra-laboratory variation is low. However, for a laboratory to routinely adopt HPV testing using a system such as current hybrid capture technology, it is necessary to achieve, and maintain, some level of expertise in this medium-level technology. Moreover, PCR-based technology requires additional skills in DNA processing and facilities with safeguards and precautions to avoid specimen contamination.

6. The validity of the currently available HPV tests in identifying HSIL and higher-grade cancers. Standard testing systems have been shown to perform with higher sensitivity than expert cytology in detecting prevalent CIN3 / HSIL or higher-grade cancers. In contrast, the specificity of the HPV test is lower than cytology in young age groups (<30), though it is comparable in the older age groups. The sensitivity and specificity of HPV testing may be lower in populations with high rates of human immunodeficiency virus (HIV) infection. The clinical sensitivity of the two most common systems available (current hybrid capture technology and PCR) is comparable.

However, there are limitations of HPV testing as a basis for screening programmes:

- The current costs of the equipment and reagents.
- The requirement to modify the paradigm of cervical screening with some implications in a) the adoption of new clinical protocols for the diagnosis and treatment of precancerous lesions and b) training health professionals.
- Transfer of the HPV testing technology to middle-income countries has to be proven and may require country-specific evaluations.

## HPV TESTING SYSTEMS: CURRENT METHODS FOR SCREENING

Before HPV testing can be recommended for use in the context of screening activities, one should be able to demonstrate that such a test fulfils the following principles:

- 1) High specificity and sensitivity for the detection of a broad spectrum of genital HPVs.
- 2) Use with minimally invasive specimens (cervical scrapes or lavage).
- 3) High level of intra-laboratory and inter-laboratory reproducibility.
- 4) Suitable for high-throughput testing.
- 5) Suitable for automated execution and reading.
- 6) Cost-effectiveness within screening programmes.
- 7) Licensed by the relevant regulatory bodies and institutions.

Additional criteria that are highly desirable for middle-income countries include short testing time and very low cost per test. Protocols that include self-sampling components may prove to be of practical value.

## TESTS AVAILABLE AND RESULTS TO DATE

At present there are two testing systems in widespread clinical use. Current hybrid capture technology has fulfilled most of the criteria above. Some consensus PCR systems that are at different stages of development also meet several of these criteria.

### Current hybrid capture technology

This test is based on hybridization in solution of long synthetic RNA probes with the HPV DNA targets present in the biological specimen (85). These RNA probes are complementary to the genomic sequence of 13 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and 5 low-risk (6, 11, 42, 43, 44) HPV types, which are used to prepare high-risk and low-risk probe cocktails, respectively. Two separate reactions are performed and the results obtained are, therefore, group-specific. For clinical purposes, only the high-risk probe cocktail is used, which reduces the time taken to perform the test and its cost.

Specimens are collected in a specifically designed transport media (STM) but can also be harvested in liquid cytology media. After cell lysis, denatured DNA from the specimen, including any HPV DNA present, is submitted to hybridization with a cocktail of RNA probes leading to the formation of specific RNA-DNA hybrids in solution. These hybrids are then captured by antibodies that specifically recognize RNA-DNA hybrids and are bound to the wells of a microtitre plate. The immobilized hybrids are reacted with a secondary RNA-DNA antibody conjugated to alkaline phosphatase and detected by the addition of a chemiluminescent substrate. Light emitted when the substrate is cleaved by the enzyme is measured in a luminometer and expressed as relative light units (RLUs), which is the ratio of light produced by each sample divided by the mean level of light produced by three positive controls (PC) of 1 pg HPV 16 DNA/ml. Different positive/negative cut-off points have been reported but in general specimens are considered positive when the ratio RLU/PC is equal to or greater than 1 (86). The intensity of emitted light is proportional to the amount of target DNA in the specimens, providing a semi-quantitative measure of the amount of HPV DNA present in the sample.

The current hybrid capture technology is configured in a 96-well microplate format with a protocol similar to an ELISA test, and, therefore, can be routinely performed by trained technicians, in clinical settings, and is also suitable for automation. Furthermore, it does not require special facilities to avoid cross-contamination, since, unlike PCR protocols, it does not rely on target amplification (HPV DNA) to achieve high sensitivity. Several studies

have reported that the current hybrid capture technology for HPV testing using the high-risk probe cocktail cross-reacts with HPV types that are not represented in the probe mix (87–89). Further studies are required to evaluate the actual false-positive rate due to cross-reactivity in different populations, the clinical impact and the reasons for such misclassification. This could be clarified by performing additional tests with the capacity to establish the HPV type involved, such as type-specific PCR-based protocols.

### **PCR-based methods**

HPV DNA can be detected by a series of assays based on the polymerase chain reaction (PCR). In this case, the viral genome is selectively amplified by a series of polymerization steps, which result in an exponential and reproducible increase in the nucleic acid sequences present in the biological specimen. Briefly, this methodology relies on the amplification of selected portions of the gene of interest whose boundaries are defined by oligonucleotides that hybridize (anneal) to their complementary sequences on the target strand that has been previously denatured. At defined temperatures, these oligonucleotides are extended by a thermo-resistant DNA polymerase leading to the formation of two new double-stranded DNA molecules (amplicon) using each of the original target DNA single strands as templates. By repeating this cycle of denaturation, annealing and extension, each newly synthesized double-stranded DNA molecule can serve as a template for the next cycle, and the number of molecules increases in an exponential fashion. Due to its exceptionally high sensitivity, care must be taken to avoid false-positive results derived from cross-contaminated specimens or reagents. Analysis of the amplified products is generally performed by gel electrophoresis, but can also be done in an ELISA format (90), and ultimately can be coupled to cycle DNA sequencing. Typing is often performed by dot blot hybridization employing multiple type-specific probes (91), restriction fragment length polymorphism (RFLP) (92) of the amplicons, or by reverse line blot hybridization (93,94).

The sensitivity and specificity of the several available PCR-based methods vary largely, depending mainly on the primer sets, 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 the ability to detect multiple types, and accessibility of a type-specific assay.

Several studies performed in the last decade employed a consensus PCR that amplifies a region of the highly conserved major viral capsid L1 gene, since it is potentially capable of detecting all mucosal HPV, with high sensitivity and specificity (91). Extensively applied generic PCR protocols make use either of the single pair of consensus primers GP5/GP6 (95) and its



extended version GP5+/GP6+ or MY09/11 degenerate primers (91). Full distinction of more than 40 HPV types can be achieved by dot blot hybridization employing multiple type-specific probes (91,96) or RFLP (92) of the amplicons obtained with the latter primers. Another very sensitive and simpler approach consists of a reverse line blot hybridization of PCR products obtained with modified MY09/11 consensus primers (93). This procedure is being adapted to run on an ELISA format making it suitable for high throughput analysis. Finally, the short PCR fragments (SPF-PCR) system generates a 65 bp fragment of a highly conserved region of the L1 gene, allowing for the detection of a broad spectrum of HPV types, by reverse line blot hybridization (96,97). In most comparisons there is good to excellent agreement between tests performed with current hybrid capture technology and generic PCR employing MY09/11 and GP5+/6+ systems (41,87,88,98–103). Some discrepancies occur between testing systems, particularly in situations requiring the identification of multiple HPV types in a given specimen and when rarer HPV types are present.

## SAMPLE PREPARATION / HANDLING

HPV detection assays have been validated with cervical samples, either cells or tissue fragments, although materials from other anatomical sites are also appropriate, provided that a minimum amount of cells or tissue is available. For some of the procedures described, different collection media can be used, including saline-buffered solutions that are inexpensive and available in any setting, and a number of product-specific transport mediums containing proprietary preservatives. Saline solutions are ideal for PCR, but depending on the location, it is desirable to have a preservative added. Specimens can be kept at 30°C for up to 2–3 weeks, but require freezing after this time, except for some of the transport mediums that cannot be frozen. Ideally, specimens should contain only the scraped cells or biopsy. However, presence of blood and mucus in tolerable amounts should not interfere with test performance. It is important to choose a collection device capable of obtaining a sufficient number of cells, without causing bleeding. However, wooden Ayres spatulas should be avoided, since too few cells are obtained and inhibition of PCR has been observed when the devices are kept in the collection medium for variable periods of time.

Although it is possible to use crude cell/tissue preparations for PCR (i.e. GP5+/6+), from almost any biological specimen including scrapes, swabs, lavages, biopsy and fixed tissues, the first step in sample preparation often involves DNA extraction. Although this is a straightforward procedure, care must be taken to assure quality, purity and in the case of PCR,

to avoid any condition that could result in contamination of the template nucleic acid. This is of lesser concern when considering current hybrid capture technology as the HPV detection test. However, for best performance, current hybrid capture technology should be run with samples containing well-preserved DNA. It is also not indicated for fixed, paraffin-embedded, tissues, since the time of fixation and type of fixative used can considerably affect the quality of extracted nucleic acids. However, for most PCR protocols, particularly with the GP5+/6+ SPF-PCR system, the latter can be considered appropriate templates, since small segments of DNA are generally expected.

To achieve high-throughput analysis and reproducibility, one should consider the use of DNA isolation devices, such as fully automated nucleic acid isolation and PCR set-up instruments. Although presently expensive, such equipment may turn out to be cost-effective in clinical settings.

## HPV TESTING SYSTEMS: DEVELOPMENTS FOR THE NEAR FUTURE

### HPV detection and typing

A highly automated device, based on current hybrid capture technology, is capable of running 700 samples per day. A third generation type-specific hybrid capture system will be available soon. This system has a lower background and increased sensitivity than current hybrid capture technology. Some of the cross-reactivity previously observed between high-risk and low-risk HPV cocktails has been significantly reduced.

A new universal collection medium has been developed in which biological specimens can be stored for up to 6 weeks at room temperature. These specimens are suitable both for HPV DNA and RNA analysis.

A generic reverse line dot assay based on amplification with PGMY09/11 primers is available with no proprietary restriction. A PCR system adapted to the conditions of the clinical laboratory is available for detection of several microorganisms including HPV. An automated system has been developed that has the capacity to process 96 samples daily.

A new test that uses an oligonucleotide set which amplifies a shorter fragment (170 bp, compared to 450 bp obtained with PGMY09/11) of the L1 gene of high-risk HPV types is also available presented. A new set of primers has been designed for high-risk types and therefore this test is not truly generic.

A new fast and reliable HPV typing method has been developed to iden-

tify individual HPV types using non-radioactive reverse line blotting (RLB) of GP5+/6+ PCR amplified HPV genotypes. In this way, 40 HPV positive clinical samples can be simultaneously typed for 37 HPV types (14HR and 23 LR types) (104). This system has been compared with conventional typing by ELISA and is evaluated using clinical samples. It could be of great value for large epidemiological studies, certain population-based cervical screening programmes and vaccination trials that require reliable HPV typing.

### **HPV viral load**

Viral load determination is under active research to evaluate whether high copy numbers are correlated with increased risk of developing HPV-associated cervical lesions (103). HPV DNA quantification in the biological sample can be achieved using PCR-based methods (98,105) or by hybrid capture technology (106). It is possible that protocols based on real-time PCR (107–110) will help to clarify the relevance of viral burden for cervical disease. It has also been reported that real-time PCR can be used to distinguish between the two most common high-risk HPV types, by exploring their melting profiles (111).

### **Other biomarkers of neoplastic transformation**

Klaes et al. (112) exploited the interference of the high-risk HPV early protein E7 in the function of pRB, which leads to over-expression of p16INK4A, a cyclin-dependent kinase inhibitor involved in cell cycle control. A simple immunohistochemistry assay has been developed that detects p16 expression in both cell smears and tissue sections.

New tests to study the expression of E6 and E7, in which linear detection of mRNA of these early genes can be obtained, are being developed.

Detection systems using microarray technology are under investigation. Alternative targets to HPV, such as telomerase gene expression, and other host genetic targets, alone or in combination with HPV testing, are also under investigation.

## **USES OF HPV TESTING IN SCREENING**

The evidence available on the value of HPV tests as a screening method relies on three types of studies:

1. Epidemiological studies focused on the evaluation of the association between HPV and cervical neoplasia. Natural history studies (follow-up

of women being regularly tested for HPV DNA and cytology) allow estimates of the sensitivity, specificity and predictive values of HPV DNA and HPV-related biomarkers in relation to CIN3/HSIL lesions. Case-control studies comparing women with and without cervical cancer in relation to their HPV exposure provided estimates of the risk of invasive cervical cancer subsequent to HPV DNA exposure.

2. Studies in which HPV testing is evaluated as a primary screening tool. Most of these studies have added or compared HPV testing to cytology in women that were part of cytology-based screening programmes. Other studies have evaluated HPV tests in women without previous cytology in developing countries.
3. Studies in which HPV tests have been evaluated as a secondary screening method to triage women with uncertain cytological results (ASCUS/LSIL). These studies have been conducted in populations with good cytology-based screening practices.

## NATURAL HISTORY STUDIES AND CASE CONTROL STUDIES

HPV infections are among the most common sexually transmitted conditions. The prevalence of high-risk human papilloma virus (HR-HPV) in woman with morphologically normal smears is age-dependent. In all populations investigated, HPV DNA prevalence reaches a peak in young women (i.e. age below 25) and spontaneously decreases to a level of 2–5 % in women over 35 years. The point prevalences observed are country-dependent and are strongly related to the dominant sexual behaviour patterns (113-116). Some of the identified determinants of HPV prevalence are the age of women at sexual initiation, the lifetime number of partners of women and of their male sexual partners, and the frequency of sexual contacts between men and prostitutes. Possible determinants of HPV prevalence include the age of men at sexual initiation, the age differences within couples at first marriage and the prevalence of male circumcision. In some populations, a second mode in HPV prevalence is observed in older women (100,117).

The prevalence of HR-HPV in specimens of invasive cervical cancer is greater than 90 % in a worldwide series tested by PCR (118) or equivalent technology. When corrections are made for non-representative tissue and inadequate DNA, it was shown that 99.7% of all carcinomas harbour HR-HPV (119).

HR-HPV is present in at least 70% of CIN1 lesions, 80% of CIN2 lesions and 96% of CIN3 lesions. Under the Bethesda system nomenclature, HPV-DNA can be identified in some 50% of ASCUS specimens, 80% of LSIL

and 90-95% of HSIL and invasive cancer cases. Variability across studies is largely related to variability in the cytological classification of the lesion (41,96,114,120).

In follow-up studies of women without cervical abnormalities, the continuous presence of HR-HPV is necessary for the development, maintenance and progression of progressive CIN disease (121-124). Clearance of HR-HPV is associated with regression of CIN lesions (125,126).

Approximately 4% of women aged 30-60 years with normal cytology at recruitment will be HR-HPV DNA positive, and 8-10% of these women will develop CIN3 lesions within the subsequent 4-years (127,128). Conversely, HR-HPV DNA-negative women, with cytology identified as either ASCUS, borderline or mild dysplasia, are unlikely to develop CIN3 during a two-year follow-up. Likewise, women who screen positive for low-risk HPVs rarely become persistent carriers, and their probability of progression to HSIL is extremely low (59,125).

Retrospective studies of women with cervical cancer show that the same HR-HPV type can be isolated in the preceding abnormal and normal smears for up to 15 years (129-130). From these studies, it follows that the time to develop cervical carcinoma after a cervical HR-HPV infection in a woman with a morphologically normal smear is at least 10 years and probably longer.

Case-control studies in different parts of the world, notably in countries with high incidence of cervical cancer, have reported risk estimates in the range of 50-fold to 100-fold for HPV DNA and cervical cancer. Risk estimates for specific associations (i.e. HPV 16 and squamous cell cancer and HPV 18 and cervical adenocarcinomas) range between 100 and 500. These estimates lead to calculations of attributable fractions of approximately 90% (131). Moreover, following an evaluation of the reasons for non-detection of HPV-DNA in some cervical cancer specimens in over 22 countries (119), it was postulated that HPV was a necessary cause of cervical cancer and, therefore, that the attributable fraction (AF) should be considered 100%.

Some 15 HPV types appear to be involved in over 95% of cases of cervical cancer. HPV 16 and 18 are the most common types identified and represent some 50% and 10% respectively of the viral types involved. There are, at present, no data to suggest that the risk of developing cervical cancer is significantly different for each of the different HR-HPV types (132-136). Studies on HPV variants (variation within HPV types affecting down to one nucleotide of the viral genome), however, are beginning to unveil risk differences (137,138). The geographical distribution of HPV variants and its relevance for HPV testing and for vaccine development is still uncertain.

Case-control studies also provide evidence on the impact of other risk factors in the promotion of HPV infections to cervical cancer. Consistent

associations have been described between long-term use of hormonal contraceptives and cervical cancer among women chronically exposed to HPV (139). A similar observation has been made for high parity (140), cigarette smoking and exposure to other sexually transmitted diseases (STDs) (*Herpes simplex virus* type 2 and *Chlamydia trachomatis*) (141). Co-infection with the human immunodeficiency virus (HIV) has also been recognized as a risk factor (142).

Studies investigating invasive cervical cancer have provided a rationale for using HPV tests in screening. Studies on advanced pre-invasive cervical neoplasia (carcinoma *in situ* / CIN3 / HSIL) have also shown results consistent with cervical cancer studies (100,127,128,143–150).

## HPV TESTS IN PRIMARY SCREENING PROGRAMMES

If HPV DNA testing can be proven to have acceptable performance characteristics as a screening test in middle-income countries, screening based on molecular testing may prove easier to implement and sustain than cervical cytology in such countries.

## HR-HPV TESTING AND HPV TYPE-SPECIFIC TESTING PERFORMANCE IN SCREENING

The risk of developing cervical cancer and the prognosis is similar for the different HR-HPV types, and it is accepted that test formats that detect the known HR-HPV types in a cocktail mix are suitable for screening. Individual typing is only necessary in research settings and for studies evaluating therapeutic or preventive type-specific HPV vaccines (101,104).

Ideally, HPV screening tests should result in the detection of all progressive CIN3 / HSIL lesions and cervical cancers. Both current hybrid capture technology and GP5+/6+ PCR/EIA have cross-sectional sensitivities for CIN3 and cervical cancer that are at least equal to, and in most studies significantly better than, cervical cytology (Table 3), (59,124,151,152). The specificity of HPV tests is age-dependent. In the young age groups specificity is lower than cytology and, in the 35 and above age groups (also country-dependent), the specificity of the tests is similar. Recent studies, in which current hybrid capture technology and GP 5+/6+ were used, showed that both tests have a high negative predictive value for CIN and cervical cancer (41,124,126,151,152). In combination, women with both normal cytology and absence of HPV DNA have an extremely low risk of developing cervical cancer in the 10+ subsequent years. Major gains in effec-

tiveness and cost reduction are, therefore, to be expected from the resulting opportunity to increase intervals between screens.

A number of studies have evaluated the performance of HPV DNA testing as a screening test including some studies in low- to middle-income countries (79,86,153–155). Most of these studies have utilized the hybrid capture HPV DNA assay and have focused on the detection of high-risk types of HPV, as defined by the assay.

Recent modelling based on data from South Africa suggests that VIA or HPV-DNA tests may offer attractive alternatives to cytology-based screening programmes (55).

## IMPACT OF HIV STATUS AND AGE ON PERFORMANCE OF HPV DNA

The comparison provided in Table 3 between cervical cytology and HPV DNA testing may not be applicable to all locales and age groups of women. For example, in the study of Kuhn et al. (86) 7% of the women were HIV seropositive and in the study of Womack et al. (154) 52% of the women were HIV seropositive in the subset of women who agreed to HIV testing. Rates of HPV DNA detection in women lacking clinical evidence of cervical lesions are 2–4 times greater in HIV-infected, compared to HIV-uninfected, women. Therefore, the specificity of HPV DNA testing might be expected to be lower in populations with high rates of HIV seropositivity. In the study of Womack et al. (154) the specificity of HPV DNA testing for the detection of HSIL /cancer was 41% in HIV-seropositive women and 75% in HIV-seronegative women. There were also age differences in the women enrolled in the studies, which might also influence the performance of HPV DNA testing. For example, Kuhn et al. (86) included women between the ages of 35 and 65 years, Belinson et al. (155) included women 35–45 years of age, and Schiffman et al. (153) enrolled women 18 and older.

Table 4 presents the prevalence of HPV DNA positivity in women stratified by age from various studies. The results suggest that in women over the age of 35 years, the specificity of HPV DNA testing in populations without high rates of HIV seropositivity will be similar to that reported for cytology.

## HPV TESTS IN THE TRIAGE OF MINIMAL CERVICAL ABNORMALITIES

One of the first applications of HPV testing was for the triage of women

Study	Country	Test	HPV DNA		Cytology	
			Sensitivity	Specificity	Sensitivity	Specificity
Kuhn et al. (86)	South Africa	HC 1	73	88	78	97
Schiffman et al. (153)	Costa Rica	HC 2	88	89	78	94
Belinson et al. (155)	China	HC 2	98	85	94	78
Womack et al. (154)	Zimbabwe	HC 2	81	62	44	91
Schneider et al. (116)	Germany	PCR	89	94	20	99
Wright et al. (157)	South Africa	HC 2 ss.	66	83	—	—
		HC 2	84	85	61	97
Cuzick et al. (156)	U.K.	HC+PCR	95	95	79	99
Ratnam et al. (158)	Newfoundland	HC 2	68	91	27	96

Table 3. Performance of HPV TESTS for detection of HSIL / cancer in selected studies that mimic population-based screening conditions

ss.: self sampling

Author – Country	Test	% HPV DNA Positive by Age				
		< 25 yrs	25–34 yrs	35–45 yrs	45–55 yrs	>55 yrs
Jacobs et al. (114) – The Netherlands	PCR	13%	10%	2%	2%	
Herrero et al. (100) – Costa Rica	HC 2	10%	6%	3%	3%	
Ratnam et al. (158) – Canada	HC 2	17%	12%	5%	4%	
Cuzick et al. (159) – U.K.	PCR		3%	3%	5%	
Clavel et al. (106) – France	HC 2	21%	20%	13%	11%	
Womack et al. (154) – Zimbabwe	HC 2		32%	22%	24%	
Schneider et al. (116) – Germany		15%	9%	6%	3%	
Lazcano-Ponce (117) – Mexico		— 12.8% —		— 7.1% —		19.3%

Table 4. Prevalence of HPV DNA detection by age group in women enrolled in screening programmes

HC1: Hybrid capture I  
 HC2: Hybrid capture II  
 PCR: polymerase chain reaction



with abnormal cytology for colposcopy. In the USA, concerns about mal-practice litigation have led to an aggressive approach that includes immediate colposcopy, repeat cytology, and the use of HPV tests for the triage of cases of low-grade disease.

In countries where the Bethesda system has not been adopted, the results from triage studies in developed countries have uncertain application. However, a significant majority of the laboratories in middle-income countries may encounter such difficulties and local studies that evaluate local expertise and test reproducibility are of importance.

The best evidence on the role of HPV testing as an alternative method to repeated cytology in the presence of an ambiguous abnormal (ASCUS) cytology has been provided by the Kaiser Permanente study and the ALTS trial. These studies have been conducted under controlled conditions in different settings within the USA.

## THE KAISER PERMANENTE STUDY

The Kaiser Permanente study used concomitant testing focused on 995 ASCUS cases among a population of 46 000 women participating in a health maintenance programme (59). HPV testing by current hybrid capture technology of the residual fluid collected for liquid-based cytology was compared with repeat cytology (at an ASCUS threshold) in identifying women with HSIL on histology. The triage algorithm considered a second cycle of reflex HPV testing after six months on persistent ASCUS smears. The proportion of women that would have been referred for colposcopy was comparable using the two approaches: 40% for HPV and 39% for repeat cytology. The sensitivity to detect HSIL or cancer was 89% for HPV and 76% for cytology, with equivalent specificities for both tests (64%). Besides having a large sample size and a defined screening cohort base, this study also has the advantage of having used several clinics and pathology laboratories, thus providing realistic triage conditions prevailing in the USA.

## THE ALTS TRIAL

The ALTS trial was a randomized trial designed to separately determine the optimal management plans for LSIL and ASCUS cytological abnormalities. It accrued 600 women with LSIL, and approximately 3 500 women with a recent diagnosis of ASCUS, from four clinical centres. Women were randomly assigned to one of three management arms: 1) immediate colposcopy; 2) conservative management and referral to colposcopy if enrolment

or any of the follow-up cytologies showed HSIL or worse, and 3) HPV (current hybrid capture technology) triage, which consisted of a colposcopy referral if the enrolment HPV test was positive or missing or any cytology was HSIL+.

The HPV triage arm of the LSIL component of the trial was terminated prematurely because of an interim observation of a high proportion of oncogenic HPV positivity (83%) (with high-risk types) among women with an entry smear of LSIL. This resulted in a high rate of colposcopy referrals, thus approximating the conditions of arm 1. The authors concluded that there was limited value in using HPV testing in triaging such women for colposcopy (41).

In the ASCUS component of the trial, HPV testing yielded 96% sensitivity to detect both CIN2+ or CIN3+ histologically-confirmed lesions, while repeat cytology at the lowest threshold of ASCUS produced a sensitivity of 85% for both definitions of lesion severity. The proportion of women referred for colposcopy was 56% and 59%, respectively. Thus, it was concluded that HPV testing was a viable option in the triage and management of ASCUS smears (120).

The Kaiser Permanente study and the ALTS trial have provided evidence of the role of HPV testing using hybrid capture (HC) technology as a triage tool for ASCUS smears in high quality laboratory conditions typically found in developed countries. Issues of cost-effectiveness have only been partially addressed by these studies, but preliminary analyses indicate that HPV testing may be superior to management by immediate colposcopy.

In a small, Canadian randomized trial of the cost-effectiveness of HPV testing *versus* repeat cytology in women with low-grade cytology, Lytwyn et al (160) demonstrated that compared to the base case of repeating conventional cytology, the incremental cost of HPV testing with hybrid capture II was approximately \$US 2000 for each additional woman identified with high-grade disease. A practical consideration raised by the Canadian trial, and hinted at in the ALTS results, is that higher loss to follow-up might be associated with the strategy of repeating cytology.

Meta-analyses of studies conducted internationally have provided some evidence that HPV testing may play a useful role in the secondary triage of minor-grade abnormalities (156). A key issue is the proportion of LSIL cases that are positive for HPV high-risk types. No other triage studies have found as high a positivity as the ALTS trial (83%). This indicates that the extensive quality control safeguards that were implemented for the ALTS trial in defining cytology categories for referral may have made the trial non-generalizable with respect to other settings. For example, the quality control panel in the ALTS study accepted only 30–60 % (average 50%) of the ASCUS diagnoses of the participating centres as ASCUS. Further, it is

likely that LSIL abnormalities as currently defined in the USA in specialized centres do not represent a category that is interchangeable with the LSIL, mild dysplasia or mild dyskaryosis analogues reported in smears read elsewhere.

Thus, it is not possible on the basis of the current evidence to make a recommendation for triage of these abnormalities using HPV testing in middle-income countries. Rather, such countries must consider the available options for triaging women with cytological abnormalities and will have to build their own knowledge base from translational studies to guide policy decisions.

## OPPORTUNITIES FOR SELF-SAMPLING IN SCREENING AND TRIAGE

Self-collected vaginal samples are alternatives to clinician-collected cervical samples for STD testing of both *Chlamydia* and *Trichomonas* (161-163). Several studies have now evaluated the possibility of utilizing self-collected vaginal samples for HPV DNA testing (157,164,165). Although the results of these studies have been promising, in several, a reduction in sensitivity for the detection of HSIL/cancer has been observed using self-collected compared to clinician-collected samples.

In a study of 200 women enrolled from colposcopy clinics, the sensitivity of current hybrid capture technology for detection of HSIL was 0.98 when performed on clinician-collected samples and 0.86 when performed on self-collected samples (165). Wright et al. (157) found that when compared in a screening setting, the sensitivity of current hybrid capture technology for HSIL/cancer was 0.84 when performed on clinician-collected samples compared to 0.69 for self-collected samples. However, in countries where trained professionals may be lacking, and cultural barriers to health professional examinations are present, self-sampling may prove to be useful in HPV-based screening programmes.

## HPV IN QUALITY CONTROL OF CYTOLOGY-BASED SCREENING PROGRAMMES

HPV DNA testing could potentially be utilized to provide ongoing quality control in cytology laboratories. There are now data from several studies documenting the prevalence of high-risk HPV DNA when assayed using the current hybrid capture technology HPV DNA assay among women with a spectrum of cytological abnormalities (Table 5).

Study	% HPV DNA Positive (range)	
	ASCUS	LSIL
Ferris et al. (170)	63%	72%
Manos et al. (59)	40%	–
ALTS (41)	–	–
Solomon et al. (120)	51% (31-60%)	83% (79-86%)
Cox (166)	37%	–
Wright (167)	42%	–
Chesebro (168)	–	80%
Fait (169)	16%	23%
Bergeron (171)	43%	58%
Fait (172)	24%	26%
Lin (173)	53%	76%
Rebello (174)	41%	75%

Table 5. Prevalence of high-risk HPV in women with ASCUS or LSIL Pap smears

	Cytology smears	Liquid cytology	HPV DNA Testing
Dependence upon sampling	highly dependent	dependent	less dependent
Use of specimen	once	multiple	multiple
Reading	subjective	subjective	quantitative scale
Quality Control	second reader	second reader	repeat test
Training	months	months	weeks
Automation	slow progress	relatively advanced	advanced
Yield	250 / person / week	350 / person / week	600+ / person / week
Cost (trend)	moderate	high (down)	high (down)
Status	standard	under evaluation*	triage (FDA)

Table 6. Comparison of test characteristics

\* some 50% of the cytology currently used in the US in 2001

The wide range of HPV DNA positivity among women referred with a diagnosis of ASCUS in the ALTS study (120), reflects the variability between laboratories and cytopathologists in the criteria used to make a diagnosis of ASCUS. As a consequence, there is currently no universally accepted expectation for the proportion of ASCUS and LSIL smears likely to be HPV DNA-positive. Moreover, in settings where liquid-based cytology is not being used routinely, it may be difficult for the laboratories to obtain the second samples required for HPV DNA testing from patients with ASCUS or LSIL. It is unrealistic to establish HPV DNA testing solely for quality control purposes in settings where it is not already being used.

## RECOMMENDATIONS ON THE DESIGNS OF STUDIES ON HPV TESTING FOR CERVICAL SCREENING

The ALTS and Canadian trials assessed the value of HPV testing as a strategy in the triage of ASCUS and LSIL using a randomized controlled trial (RCT) design, whereas all other published studies have resorted to a concomitant, split-sample testing design. Likewise, all published studies of HPV testing in primary screening have been of the latter kind. Future studies should rely on the RCT design to produce the weight of evidence needed for policy recommendations. Although adequate as proof of principle, concomitant testing designs do not allow assessment of long-term outcomes and do not represent real screening conditions.

A critical element for evaluation trials is the choice of outcome. Primary screening studies have relied on a cross-sectional assessment of prevalent lesions to estimate diagnostic efficacy (sensitivity and specificity). Focusing on histologically-defined, prevalent HSIL is an adequate strategy to obtain proof of principle that HPV testing may fare as well as, or better than, cytology. However, this proof is not unequivocal because HPV testing may differ qualitatively from cytology with respect to the detection of lesions that are likely to progress to cancer. If prognostic differences exist between the subsets of HSIL that are detected by HPV and cytology, the gain in screening yield may not translate into reduced incidence of cervical cancer if HPV testing was adopted. In consequence, the definitive proof that HPV testing may represent an improvement over existing cytology programmes can only be obtained by demonstrating a reduction in the incidence of invasive disease. Because of the rarity of invasive cancer, this would require large trials. The need to use invasive cervical cancer as an endpoint may seem to create an ethical dilemma, because RCTs with active monitoring of lesions cannot be conducted without treating all identified prevalent and incident HSILs. However, since the standard of comparison will be cervical cytology,

or in some settings VIA, the precise answer required will always be obtained. These large RCTs cannot use CIN3 as the primary outcome, as this is one of the lesions that cytology is designed to detect, and its detection and treatment must be regarded as a success rather than a failure of screening. Therefore, although in all arms every means must be taken to detect and treat precursor lesions and prevent invasive cancer occurring, it is already known, that at least for cytology (the current standard of care), invasive cancers will occur. Hence, the incidence of cervical cancer will have to be determined by active or passive follow-up via record linkage with tumour registries and mortality databases.

It is also important in ensuring that trials are relevant for future policy decisions that the screening tests being compared are analyzed in the same countries as the specimens are collected.

Conventional RCT's in which HPV testing is offered to one study group and cytology, as the standard option, to the reference arm are preferred. However, alternative approaches may be needed in areas where no cytology facilities are available, or cannot be organized to cope with the requirements of one of the branches of such large trials. There are also less rigorous study designs that could be used if randomization is deemed unacceptable. In general terms, these could adopt the format of either:

1. Quasi-experimental, population-based, designs in which HPV tests are offered to a large population without current access to cytology-based programmes. Incidence/mortality of invasive disease is then compared to like populations without such an intervention.
2. Time trend monitoring (before and after) of cervical cancer incidence/mortality which is then related to the intake and penetration of HPV testing.

Another required direction for HPV screening studies is the evaluation of efficacy and cost-effectiveness of multiple HPV testing modalities and sampling strategies. Most published studies have relied on the hybrid capture assay, which is the only test approved by the FDA. Other competing HPV tests, based on PCR or other techniques, may soon become available commercially. Little is known about their performance in screening conditions. Also desirable are studies that evaluate strategies that aim at simplifying the screening process, e.g., self-sampling, use of urine specimens, etc. These strategies will need to be assessed in both resource-rich and resource-poor settings, as the results may vary substantially due to differences in the prevalence of STDs and to cultural differences in accepting active patient participation in the screening process.

## ADVANTAGES AND DISADVANTAGES OF HPV TESTS IN SCREENING

Despite the fact that HPV DNA testing has performance characteristics that make it an attractive alternative to cytology as a primary screening method in women over the age of 35 years in populations with a low rate of HIV seropositivity, it remains unclear whether HPV DNA testing will prove easier to implement and sustain than cytological screening. Table 6 illustrates some of the features that may help in making decisions concerning the adoption of HPV tests. Equivalent features for liquid-based cytology have been added for comparative purposes.

The advantages of HPV DNA testing are that it does not require the same level of technical expertise as cervical cytology; it is amenable to large-scale, robotic processing in centralized laboratories; quality control measures are directly incorporated into the testing method; it identifies women with current disease and those at risk for developing disease over the next 2–3 years; and it can be performed on self-collected vaginal samples, eliminating the need for a speculum examination, albeit with some loss of sensitivity. The disadvantages of HPV DNA testing are its high costs; dependence on reagents currently produced by only a single commercial manufacturer; requirement for a molecular diagnostic laboratory and its low specificity in younger women and populations with significant rates of HIV seropositivity. More importantly, since HPV DNA testing, like cytology, is not a test that provides results at the time of the visit or soon afterwards, many of the traditional barriers to cytological screening remain. These include the need to transport specimens to a laboratory for evaluation, and the need to recall women to undertake diagnostic tests and provide treatment.

## CONCLUSIONS ON HPV TESTING

Accumulated evidence on HPV testing shows that it is an acceptable, safe and effective procedure for detecting cervical cancer precursors at a sufficiently early stage to permit intervention. In addition, current technology is suitable for large population-based screening trials.

However, at present, no population-based, randomized trial comparing HPV testing to standard cytology in primary screening has been published. Furthermore, the studies that allow cross-sectional estimates of the sensitivity and specificity of HPV tests to be compared to cytology in general compared HPV testing to high quality cytology rather than to standard cytology and were performed under diagnostic, rather than screening, circumstances.

In such studies, the sensitivity to detect HSIL or cervical cancer was greater for HR HPV tests than for standard cytology. The increase in sensitivity is country-dependent. Specificity in the age groups of age 30 and above is comparable between cytology and HPV testing. The negative predictive value of a HPV test is between 90 and 99%. The negative predictive value of cytology and HPV tests combined is between 97 and 99%. These results are applicable to populations in which cytology programmes are in place. Middle-income countries may benefit from these results in the management of women with access to cytology-based programmes. However, the applicability of these results to screening programmes is limited.

The major logistical advantages of HPV screening include the ability to automate the testing procedure and to generate large volumes of results per person time unit at a standard level of quality. The procedure allows for HPV testing to be conducted in a highly centralized manner. Sampling-dependency and quality control is also favourable for HPV testing as compared to cytology.

The most important limitations in adopting HPV tests as the primary screening tool in populations with sub-optimal cytology coverage are:

1) The lack of unequivocal population-based evidence as to the effectiveness of the HPV test as a stand-alone screening tool; 2) the current high costs of the equipment and reagents and 3) the necessity to change an established paradigm for cervical screening.

In middle-income countries with some laboratory skills and limited impact of cytology-based screening practices, HPV DNA tests as the primary screening test may offer an alternative for the reduction in incidence of cervical cancer. Ongoing research should provide data on the cost-benefit balance of screening programmes that adopt HPV as a stand-alone screening test. Final proof of the capacity to reduce the incidence of cervical cancer can only be provided by carefully conducted intervention trials.





## OVERALL CONCLUSION

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# 6

In conclusion, cytology screening remains the standard for application in middle-income countries. However, VIA holds substantial promise, and providing this is confirmed in the ongoing studies, and the difficulties associated with its lower specificity overcome, it may replace cytology in lower income countries. Tests for HPV DNA have shown efficacy in the triage of equivocal diagnosis (ASCIS in the Bethesda system) and, if costs and technology are made affordable, could eventually become the gold standard. All tests, however, have to be applied within an organized setting, preferably as a component of a National Cancer Control Programme.



## *APPENDIX I:*

# **Epidemiological Issues in the Evaluation of Alternative Screening Tests**

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### TESTS USED IN THE DIAGNOSIS AND MANAGEMENT OF MINIMAL CERVICAL ABNORMALITIES

Most studies testing triage (for diagnosis and management) have been based on a concomitant testing design, i.e., all women meeting the enrolment criteria undergo the alternative test and a repeat cytology. Studies of this kind collect information on both tests and obtain histological assessment of all true lesion cases among the referral sample (gold standard), which permits the computation of cross-sectional sensitivity, specificity, and positive and negative predictive values. There has been great enthusiasm concerning the use of HPV testing and other adjunctive techniques in such situations because of the apparent improvement in diagnostic yield of lesions, giving the impression that combined testing has greater sensitivity than cytology alone. However, this increase in sensitivity can be misleading, even if it is statistically significant. Combined testing prevents a loss in specificity but sometimes offers no real sensitivity gain. A nominal increase in sensitivity always occurs by chance whenever an adjunct test is used in parallel with a conventional one, even if the new test was totally random with respect to the disease being evaluated (175,176). Gains in sensitivity and losses in specificity need, therefore, to be gauged against the expected levels of these parameters assuming that cytology is augmented by a random adjunct test and not against the sensitivity and specificity of cytology alone (175). When correction for this bias was made for some of the reports on triage using HPV testing some of the apparent gains in sensitivity lost statistical significance. Correspondingly, the reduction in specificity that resulted by combining cytology and HPV tests was always significantly greater than the specificity expected by the addition of a second random test (175).

### INVESTIGATIONS OF TESTS IN PRIMARY SCREENING

Investigations of tests for primary screening are complex and expensive because of the large numbers of subjects to be tested before a sufficiently high number of lesion outcomes can be accrued to allow statistical precision in computing screening performance indices. It thus becomes impractical

to ascertain the presence of disease by colposcopy and biopsy in all women entered into the study. It is also ethically questionable to submit all low-risk, asymptomatic women who tested negative at screening to a set of diagnostic procedures that carry some degree of risk. As a consequence, because the gold standard measurement (colposcopy and histology) is extended only to those with a positive result, it becomes impossible to directly assess sensitivity cross-sectionally. There are, however, indirect estimation methods that allow computation of diagnostic parameters when more than one test is applied (one being the gold standard) and disease confirmation is restricted to positive cases (36). However, to yield unbiased estimates, such methods require the important assumption that the errors of the two tests are not correlated, which in practice cannot be guaranteed. Another solution is to obtain the gold standard measurement in a statistically informative random sample of the women with a negative screening test. However, this does not prevent verification bias entirely unless additional corrections are made (158,177,178).

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Of the 25 experts who participated in this Consultation, one expert declared an interest in the subject matter. Dr Thomas Wright of Columbia University received research funds and speaker fees from two companies which manufacture diagnostic tests for cervical cancer, that are discussed in this report.





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Cervical cancer is the second most common cancer among women worldwide, with almost half a million new cases each year. Almost 80% of the women affected are in the developing world. However, many of these cases could be prevented from progressing to invasive disease, and potentially death. More so than any other cancer, cervical cancer is a disease which lends itself to early detection and treatment. The effectiveness of cytology screening as a method to reduce the number of invasive cases and deaths resulting from cervical cancer in developed countries has already been demonstrated. Alternative screening tests, such as Visual Inspection with Acetic acid (VIA) and Human Papilloma Virus (HPV) are currently being examined and may prove feasible in the near future.

Policy makers and clinicians are faced with the responsibility of establishing and reviewing screening programmes that have the potential to save the lives of many millions of women each year.

This report offers a summary of the evidence on which to base important decisions. It focuses particularly on the situation in low and middle income countries — countries in which cervical cytology screening may not be feasible or cost-effective. It documents the current state of evidence concerning alternative tests — VIA and HPV testing. It reviews trials that are currently being undertaken, and gives policy makers an indication of developments that are likely to emerge in the near future. However, it also emphasizes that the efficient and effective functioning of the system in its entirety is central to the success of any screening programme, irrespective of the screening method chosen.

This publication is the product of a comprehensive consultation undertaken by WHO in 2001 and subsequent discussions which continued for several months after the meeting, involving leading experts in the fields of cancer epidemiology, screening and treatment. It is part of WHO's commitment to provide evidence-based guidelines to decision makers and highlights the priority that should be given to cervical cancer screening and treatment as an essential component of any comprehensive National Cancer Control Programme.



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ISBN 92 4 154572 0

