# An Evaluation of Alternative Strategies for the Prevention of Cervical Cancer in Low-Resource Settings

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**Thesis presented for the Degree of**
**Doctor of Philosophy**
**In the Department of Obstetrics & Gynaecology**
**University of Cape Town**
**May, 2000**

# Declaration

**PhD THESIS TITLE:**

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# List of Abbreviations

| | |
|---|---|
| ASCUS | Atypical Squamous Cells of Unknown Significance |
| CANSA | Cancer Association of South Africa |
| CHF | Community Health Forum |
| CHW | Community Health Worker |
| CIN | Cervical Intra-epithelial Neoplasia |
| DNA | Deoxyribonucleic Acid |
| DVI | Direct Visual Inspection of the Cervix after Application of 5% Acetic Acid |
| HC I and II | Hybrid Capture™ |
| HPV | Human Papillomavirus |
| HPV (1x) | Human Papillomavirus at 1x the positive control |
| HPV (10x) | Human Papillomavirus at 10 x the positive control |
| HSIL | High-grade Squamous Intra-epithelial Lesions |
| LEEP | Loop Electrosurgical Excision Procedure |
| LLETZ | Large Loop Excision of the Transformation Zone |
| LSIL | Low-grade Squamous Intra-epithelial Lesions |
| NPV | Negative Predictive Value |
| Pap Smear | Papanicoloau Smear |
| PPV | Positive Predictive Value |
| RCI | Reid Colposcopic Index |
| RLU | Relative Light Units |
| SIL | Squamous Intra-epithelial Lesions |
| TZ | Transformation Zone |
| UVI | Unaided Visual Inspection of the Cervix |
| WHO | World Health Organisation |

# Dedication

This thesis is dedicated to my parents, Mary and Hugh Denny, who are and always have been my greatest source of inspiration - with my deepest love and gratitude.

# Acknowledgements

The study presented in this thesis could not have been carried out without substantial contributions by a large number of people. In particular, I would like to express my thanks to the following people.

Dr Thomas Wright, Head: Division of Gynecological Pathology, Columbia University, New York, New York who served as a supervisor of this thesis and as one of the team that designed the study, analysed the data and who reviewed all histology. Dr Wright has filled the roles of mentor, colleague collaborator and friend – 'my angel of mercy', to whom I shall forever be grateful for bringing me this great project and for giving me the freedom to run with it.

Dr Louise Kuhn, Epidemiologist, Gertrude Sergievsky Institute of Epidemiology and Biostatistics, Columbia University, New York, New York: also a member of the team who designed and analysed the study. I would like to acknowledge her most understated brilliance and to thank her for being endlessly patient with my multitudinous queries, for reading countless drafts of the manuscript and encouraging me to push on when courage failed.

Professor Basil Bloch, President of the Cancer Association of South Africa and previous Head of the Gynaecology Oncology Unit, University of Cape Town, who first introduced me to Dr Thomas Wright. Professor Bloch was my first mentor, who taught me most of what I know today, for which I am deeply grateful.

Dr Robbert Soeters, Head of the Gynaecology Oncology Unit, Department of Obstetrics & Gynaecology, UCT, also a supervisor of this thesis, who did everything in his power to support me through the rigours of writing this thesis.

Professor Peter Cruse, Head: Department of Anatomical Pathology, UCT, a supervisor of this thesis and who gave me free access to the services of his department and constant encouragement.

Dr Harry Jordaan: For reading the first draft of the manuscript and for encouraging me to believe in myself.

Mr Freddie Lotters and Mr Keith Rossouw who both made countless trips to the library to fetch and photostat articles for me - I could not have done this work without them.

Patricia Papier and Anneli Visser who performed the HPV DNA testing in between busy work schedules.

My project team: Sister Budgie Panyeko (who screened the first 2944 women), Mrs Johanna Maluka (Enrolment), Mrs Thandiwe Batyi (Recruitment, Tracing and Education), Mrs Sindiswa Baraza (Driver, Recruitment, Tracing and Education), Dr Liliana Risi and later Sister Nocawe Kuse, Ms Nosipho Yeza, Sister Noxolo Wayeza and Dr Simone Honikman – a team of women dedicated to excellence, combined with compassion, gentleness and humour. My appreciation and respect for each one knows no bounds.

Professor Van Der Spuy and the consultants in the Department of Obstetrics & Gynaecology, University of Cape Town, particularly Dr James Nevin of the Gynaecology Oncology Unit, who generously gave me the space, encouragement and support to do this work.

The women of Khayelitsha who participated so generously in this project and from whom I learned so much.

Finally and most importantly, this work would not have matured and born fruit were it not for the constant attention, emotionally and intellectually, given to me by my partner, Dr Katrien Dehaeck.

## Funding

This project was made possible through financial and logistic support from the following:

AVSC International (Principal Funders), the Cancer Association of South Africa (mobile caravan, office space and administrative support), Department of Health, Anglo American Chairman's Fund, Rotary Constantia (who donated a car), Digene Corporation (who donated all HPV testing equipment and the HPV kits), National Testing Laboratories (who donated all Cerviscopes™ and Cervigrams™), Cooper Surgical Instruments (who donated speculums and other equipment required for LEEP) and the Medical Research Council of South Africa who entered all the data into a dataset. In addition, we received a grant from the Belgium Government to develop educational material.

# Abstract


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| **Title** | An Evaluation of Alternative Strategies for the Prevention of Cervical Cancer in Low Resource Settings |
| **Author** | Dr Lynette Denny |
| **Date** | May 2000 |

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**Introduction:** Cervical cancer is the commonest cancer cause of death among women in the developing world. This is in part due to the failure of these countries to establish cytologically-based screening programmes for the prevention of cervical cancer. This study was designed to evaluate alternative methods to cytology for screening to prevent cervical cancer in low-resource settings.

**Design:** Cross-sectional study located in a squatter settlement outside Cape Town, South Africa

**Sample:** Volunteer sample of 2944 previously unscreened women, aged 35 - 65 years, who were not pregnant, had not undergone hysterectomy or previous treatment for cervical disease.

**Methods**: Women were screened by a trained nursing sister in the following sequence: 1] A Pap smear was performed. 2] The tip of the cervical sampler was then placed in a collection tube for analysis of 9 different types of high-risk HPV DNA (Hybrid Capture 1™). In addition, a subset of all women with histological diagnosis of LSIL, HSIL or cancer and a random sample of 243 women with no histological evidence of disease were re-tested using the new generation Hybrid Capture II (HC II) test which has probes for 13 different high-risk types of HPV and has a 10-fold lower limit for the detection of HPV DNA. 3] The cervix was washed with 5% acetic acid and examined for the presence of an aceto-white lesion using the naked-eye, followed by magnification using a hand-held 2.5x lens. 4] After a second application of 5% acetic acid, two 35-mm photographs were taken of the cervix using a Cerviscope™. The photographs were processed and evaluated at National Testing Laboratories, USA. Women with a cytological diagnosis of SIL or cancer, a positive DVI examination, high levels of high-risk HPV DNA (RLU>10x positive control), or a Cervigram™ evaluation of warrants colposcopy, SIL or cancer were referred for an on-site colposcopic examination and histological sampling or treatment with LEEP (loop electrosurgical excision of the transformation zone). Women with four negative screening tests or no disease diagnosed by colposcopy and histological sampling were considered 'disease free'. Women with a histological diagnosis of LSIL, HSIL or cancer were considered 'disease positive'. To calculate sensitivity and specificity specifically for HSIL and cancer (i.e. high-grade lesions), LSIL was included in the 'no disease' group. All histology and Cervigrams™ were reviewed masked to clinical information

**Results:** Of the 2944 women screened, 3.2% (n = 95) had LSIL, 2.4% (n = 74) had HSIL, 0.4% (n = 12) had cancer, 20% (n = 579) had no disease after colposcopy and histological sampling, 71% (n = 2102) had four negative screening tests and 2.6% (n = 82) were lost to follow up. DVI identified 18.1% of women as having a positive test and detected 67% (95%CI: 56 – 77) of the cases of HSIL and cancer. A positive Pap smear was diagnosed in 8% of women and identified 78% (95% CI: 68 – 86) of the cases of HSIL and cancer. HPV DNA testing using HC I identified 6% of women as having a positive test when the higher threshold of 10 x the positive control was used (i.e. 1 million copies of HPV DNA), compared to 16% when the lower threshold was used (i.e. 1 x positive control equivalent

to 100 000 copies of HPV DNA). At the 10x cut-off, HC I identified 50% (95%CI: 39 – 61) of the cases of HSIL and cancer and at the 1 x cut-off HC I identified 73% (95%CI: 62 – 84) of the cases. HC II identified 88% of the cases of HSIL and cancer at the 1 x cut-off. By contrast Cervicography™ classified 11% of women as positive and identified 58% (95%CI: 47 – 69) of the cases of HSIL and cancer. The specificities for HSIL and cancer of DVI and HPV DNA testing at the 1x cut-off using both HC I and HC II were the lowest at 84%, 89% and 82% respectively. The highest specificity for HSIL and cancer was found for HPV DNA testing using HCI at the 10x cut-off and for cytology at 95 and 95% respectively. The specificity of Cervicography™ for HSIL and cancer was 92%.

**Conclusions**: The finding that DVI, a simple, cheap method of screening has a marginally lower sensitivity for HSIL and cancer compared to high-quality cytology, despite a lower specificity (84% versus 95% respectively) has important implications for low resource settings, where cytologically-based screening programmes have not yet been established. HPV DNA testing has considerable potential as a screening test as it is an objective test, is easier to perform than cytology and it identifies an equivalent number of high-grade lesions as cytology. In addition, it may identify women at high risk for developing SIL or cancer in the future, enabling resources to be targeted towards HPV positive women. Shifting the cut-off to define a positive test can alter the specificity of the test. Cervicography™ has relatively poor test characteristics and requires equipment that is more sophisticated than DVI. While these alternatives to cytology have the potential to replace cytology as screening tests in low-resource settings, their utility and cost-effectiveness in mass screening programmes need to be tested in prospective randomised trials.

## Overview of Study

Cervical cancer, the commonest cancer among women in the developing world, is considered a preventable disease. The disease is prevented by screening the cervix with cervical cytology for the presence of cervical cancer precursors, known as cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL). Once these lesions are detected, confirmed histologically after colposcopic assessment, treated and followed up to detect recurrence or persistence, progression to cervical cancer can be prevented in the vast majority of women. In addition, cervical screening enables detection of early, clinically inapparent cancers which if adequately treated, have an excellent prognosis compared to cervical cancers treated in advanced stages.

This model for the detection and treatment of preinvasive lesions of the cervix is considered the 'gold standard' for the prevention of cervical cancer. It is however, a relatively expensive process requiring a sophisticated health infrastructure that is rarely available in developing countries. The process requires high quality, well-equipped cytology and histology laboratories with ongoing training of staff and reliable built-in quality control. Further, the result of the Pap smear is usually delayed, necessitating a system of either tracking women with abnormal results or of ensuring that women return to the clinic for their results. Very few low resource settings have reliable methods of modern communication between patients and clinics, such as telephones, fax machines or even adequate postal services. In addition, the management of women with abnormal Pap smears may require at least 3 or more clinic visits, often to centres at considerable distance from where women live. As a consequence, instituting mass cervical screening programmes based on cytology has proved prohibitively complex for poor countries. Screening, if performed at all, tends to be opportunistic and hospital-based. Colposcopic services, if available, tend to be located in tertiary health services and are thus inaccessible to the majority of women. Further, laboratory services are limited and often unable to maintain the high quality standards required by cytology.

In the light of the failure of developing countries to institute cytologically-based screening programmes, the need to develop and evaluate alternative, more accessible methods of cervical cancer screening has become important. Developing a screening method that avoids the infrastructure required by cytology and eliminates the need for colposcopic triage may enable cervical cancer screening to be initiated and maintained in low-resource settings. Specifically, a screening process that uses mid-to low-level clinicians who are trained to perform on-site screening, diagnosis and treatment at one clinic visit may considerably simplify the process and reduce the costs involved.

This thesis will present a study, performed in a periurban squatter settlement located outside Cape Town, South Africa, which compared the performance of three different screening methods to cervical cytology. The study was performed in the context of a low-resource setting where a trained nursing sister screened all women. In addition, women with abnormalities detected on any of the four screening tests were referred for colposcopic assessment. Colposcopy was performed on-site within

days or weeks of women being screened. This enabled the alternative screening tests to be compared in the context of performing the 'gold standard' approach to cervical cancer screening.

The overall objective of the study was to evaluate new and alternative methods to cytology for cervical cancer screening, specifically in a low resource setting. The specific objectives were:

1. To compare the sensitivity, specificity, positive predictive value and negative predictive value of the following three cervical screening tests with those of cervical cytology:
    - Direct visual inspection (DVI) of the cervix with the naked eye and with low magnification after the application of 5% acetic acid.
    - Detection of high-risk of Human Papillomavirus (HPV) infection of the cervix
    - Cervicography™ (35 mm photographs of the cervix taken after the application of 5% acetic acid)
2. To analyse information on the performance of the four screening tests and the prevalence of disease in the study population to predict expected outcomes of each test used alone or in different combinations of the screening tests.
3. To determine, in a low-resource setting, the success rate, short and intermediate term complication rates of LEEP as a treatment for preinvasive disease of the cervix and to evaluate LEEP when used in a 'see and treat' mode i.e. treatment of preinvasive lesions of the cervix with LEEP after colposcopic assessment but without prior histological evaluation.
4. To describe the process of setting up a cervical cancer screening study in the context of a community-based research project.

The screening tests used in the study were:

1. **Direct Visual Inspection of the cervix (DVI):** This method of screening involves training a mid - level clinician (such as a nursing sister) to wash the cervix with 5% acetic acid and to identify aceto-white lesions, using the naked eye or using magnification provided by a hand-held magnifying lens. The nursing sister was trained to classify all aceto-white lesions as a positive test, without attempting to grade the severity of the lesion. This method of screening provides an immediate on-site result, uses minimal equipment, does not require the backup of laboratory services and can be performed in a very low technology setting.

2. **HPV DNA detection**: Using a quantitative commercial kit, known as Hybrid Capture I™ (HC I)(Digene Corporation, Silver Spring, MD), which is able to detect 9 high-risk types of HPV DNA, this study evaluated the role of HPV DNA detection as a primary screening test. Women who had high levels of detectable HPV DNA on the cervix (equivalent to a million copies of HPV DNA or 10 x the positive control) were referred for colposcopic evaluation. In addition, a subset of women with histologically confirmed LSIL, HSIL or cancer and a random sample of women with no histological evidence of disease were retested using the new generation HC II test which has probes for 4 additional types of high-risk HPV DNA and uses a micro-titre format (compared to

the tube-based format of HC I). The potential advantages of HPV DNA testing include that the test gives an objective result compared to the subjective nature of cytology. In addition, the requirements of the test are much simpler than cytology in terms of quality control and initial training of technicians and the test gives a more rapid turn around of results.

3. **Cervicography™**: This method of screening involves taking a 35-mm photograph of the cervix after the application of 5% acetic acid. The slides are then developed and read off-site by specially trained clinicians. In the study, any Cervigram™ evaluation of 'warrants colposcopy', LSIL, HSIL or cancer was considered positive and women were referred for colposcopy. The advantages of Cervicography™ are that it provides a permanent visual record of the cervix and it is easy to train clinicians to use the technique. In the context of the study, it also provided a means of quality control for DVI and a safety net to ensure that the screening sister missed no clinical cancers. However, like cytology, the result is delayed and the interpretation of the slides is subjective.

4. **Cytology**: All women had a Pap smear performed and all smears were read by the University of Cape Town Cytopathology laboratory according to the routine protocol of the laboratory and using the Bethesda system for reporting. A cytological diagnosis of LSIL, HSIL or cancer was considered a positive test and a reason for referral for colposcopy.

If any of the four screening tests were abnormal, women were referred for colposcopy. DVI results were available immediately, HPV DNA within 2 to 6 days of women being screened, cytology within 2 weeks and Cervicography™ within 8 – 12 weeks. All women were asked to return for results or colposcopic examination within 2 to 6 days of being screened.

Colposcopy was performed on-site in a specially adapted caravan, which was parked permanently at the study site. All colposcopies were performed by the author or under the direct supervision of the author. Colposcopic examination was performed using the Reid's Colposcopic Index (RCI) to grade the severity of the lesions identified and colposcopy was performed blinded to the cytology result, unless a positive Pap smear was the only reason the woman was referred for colposcopic assessment. If an insignificant aceto-white lesion was noted, a biopsy was performed and if no lesion was noted, endocervical curettage was performed. Obvious clinical cancers were biopsied. If CIN (of any grade) was diagnosed at the colposcopic examination women were treated immediately, on-site, with LEEP (loop electrosurgical excision procedure) LEEP was performed under local anaesthetic, without prior histological confirmation of the lesion – the so-called 'See and Treat' approach. Women who were treated were followed up at 4 and 10 months post-treatment with a colposcopic examination, a Pap smear and either biopsy or endocervical curettage where appropriate.

This thesis will describe the development of the screening study in the context of a low-resource setting and will present socio-demographic data and risk factors for preinvasive and invasive disease among 2944 women who were recruited to the study. In addition, the performance of the individual screening

tests are analysed and compared to one another with respect to sensitivity, specificity, positive and negative predictive values of the tests for the detection of LSIL, HSIL and cancer. Extrapolating from the performance of the screening tests, different screening strategies and their expected outcome in terms of disease detection and treatment will be discussed. Finally, the outcome of on-site treatment of preinvasive lesions with LEEP will be evaluated in terms of complications and eradication of disease. The findings of the study will be discussed in terms of a possible national screening policy and will highlight areas for future research.

# Chapter One

# Introduction

## 1.1 The Extent of the Problem of Cervical Cancer with Particular Reference to South Africa

Cervical cancer is the commonest cancer cause of death in women in Sub-Saharan Africa, South and South East Asia and Latin America. In 1980 there were an estimated 465 000 new cases of cancer of the cervix diagnosed worldwide, 80% of which occurred in developing countries[1]. In 1990, there were an estimated 360 000 new cases of cervical cancer diagnosed worldwide, with approximately 190 000 deaths recorded [2]. There is a paucity of reliable data on cervical cancer from developing countries and the ideal data (cancer incidence rates derived from population-based cancer registries) are in the main, not available [3].

South Africa launched a pathology-based cancer registry in 1986, relying on information reported by 80 private and public laboratories. In 1986, the total number of cancers reported in women was 16 559, of which 2897 (17.4%) were new cases of histologically confirmed cervical cancer (Cancer Registry of South Africa, 1986). In 1992, the total number of reported cancers in women had increased to 25 143, and the percentage of new cases of cervical cancer remained at the same proportion as was reported in 1986 at 17.8% (n = 4 467) [4]. There were 1105 deaths from cervical cancer recorded in 1992. It is acknowledged however, that a significant number of women with cervical cancer die without the diagnosis of cervical cancer being made, no histological sampling is performed or the disease is not registered [4]. This is particularly true in the rural areas of South Africa and the former 'homelands' created by the Apartheid State prior to 1994 *. This suggests that the reported number of cases of cervical cancer is an underestimate of the true number of cases.

According to the 1992 South African cancer registry, the overall crude incidence rate of cervical cancer was 23/100 000 women and the Age Standardised Incidence Rate (ASIR) was 30.5/100 000[4]. There were however, marked differences in incidence of cervical cancer in the various population groups. Cancer of the cervix was the most common cancer among black women (33.7%), second most common in coloured women (24.5%), followed by Asian women (10.3%) and finally by white women (3.5%). The ASIR of cervical cancer in black women was 34.6/100 000 with a lifetime risk (LR) of developing cervical cancer of 1 in 26 compared to an ASIR of 12.3/100 000 white women with a 1 in 83 lifetime risk of developing cervical cancer. In 1986, the ASIR of cervical cancer for black women in SA was just below the 1992 figure at 31/100 000, suggesting that the incidence of cervical cancer in South Africa has not changed appreciably over these two time periods.

* In 1994 South Africa underwent its first democratic election which terminated the policy of Apartheid by which the country had been ruled since 1948

Figure 1 shows the Age Specific cervical cancer rates per 100 000 women in the different ethnic groups in South Africa in 1992[4]. In all groups the rate remained very low until age 30 years after which the rates rose steadily to peak at between 50 and 79 years. The highest age specific rate for all race and age groups occurred among black women aged 60 – 64 years with a rate of 145.6/100 000.

The Age Specific Frequency of cervical cancer in 1992 follows a similar pattern with only 9.7% of cervical cancer occurring in women under 35 years of age[4]. The highest number of cases were diagnosed in the 50 – 54 year age group, 13.1% (n = 584), followed by the 40 – 44 age group, 12.5% (n = 560), followed by the 60 – 64 group, 12.4% (n = 555).

There is very little data on the incidence of cervical cancer in South Africa prior to the establishment of the cancer registry in 1986. However in 1960, Higginson and Oettle[5] published a survey of cancer incidence in black and coloured people in the Transvaal (one of the largest provinces in South Africa) from 1953 to 1955. Their data showed that cervical cancer was by far the commonest cancer in women, accounting for 41.7% of all cancers in black women and 37% of all cancers in coloured women. These figures corresponded to an overall crude incidence rate of 31/100 000 black women and 39/100 000 coloured women. The data were based on all histological confirmed cases of invasive cervical cancer cases in the area and included rural and urban women.

The ASIRs of cervical cancer in South Africa are similar to those of other developing countries. Figure 2 shows the marked difference in ASIRs in Southern Africa, South America and South East Asia compared to developed parts of the world such as North America and Europe, based on data published in 1985[6]. These marked differences in incidence rates between developed and developing countries have largely been attributed to mass cervical cancer screening of women.

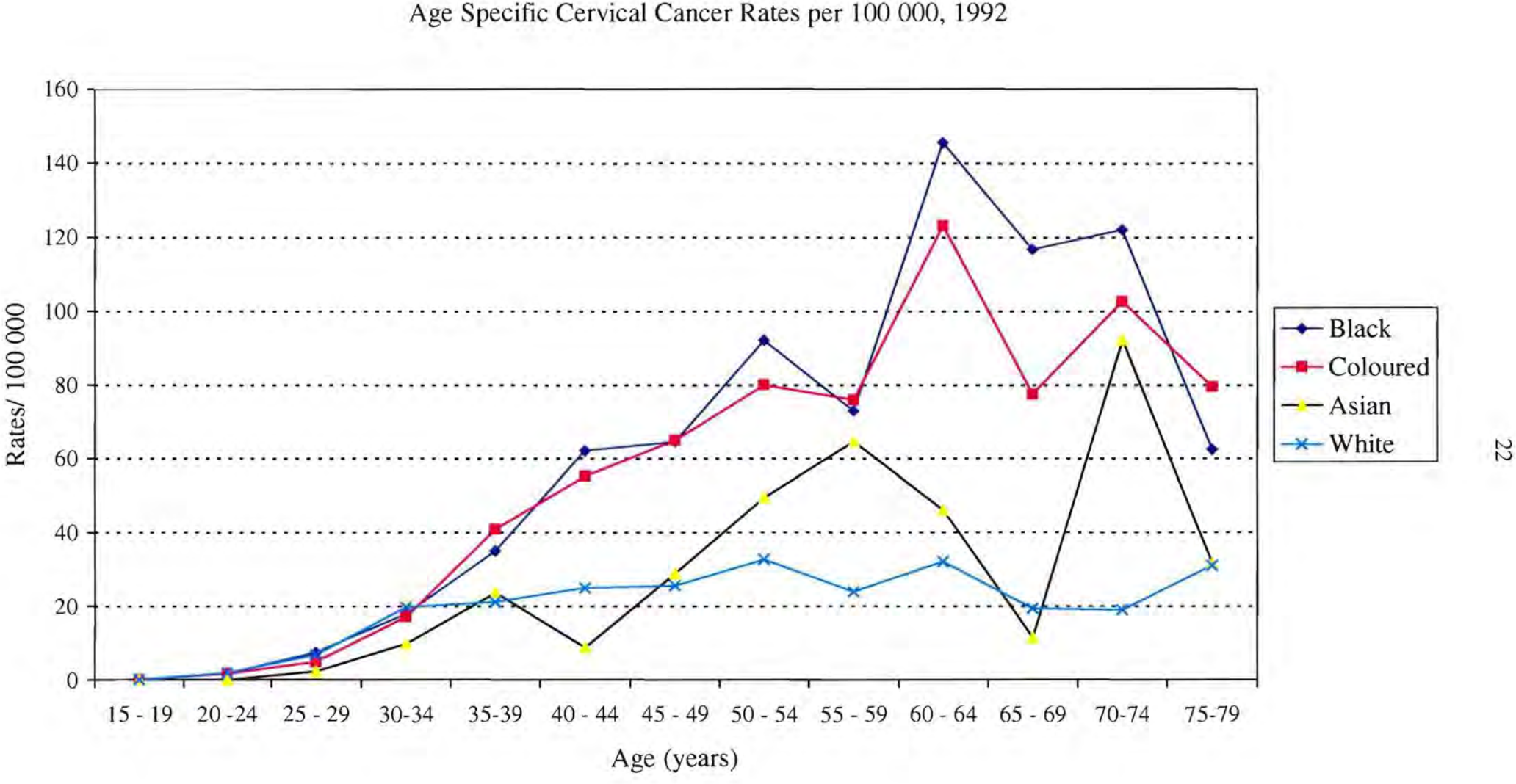


Figure 1. Age specific rate of cervical cancer in four ethnic groups

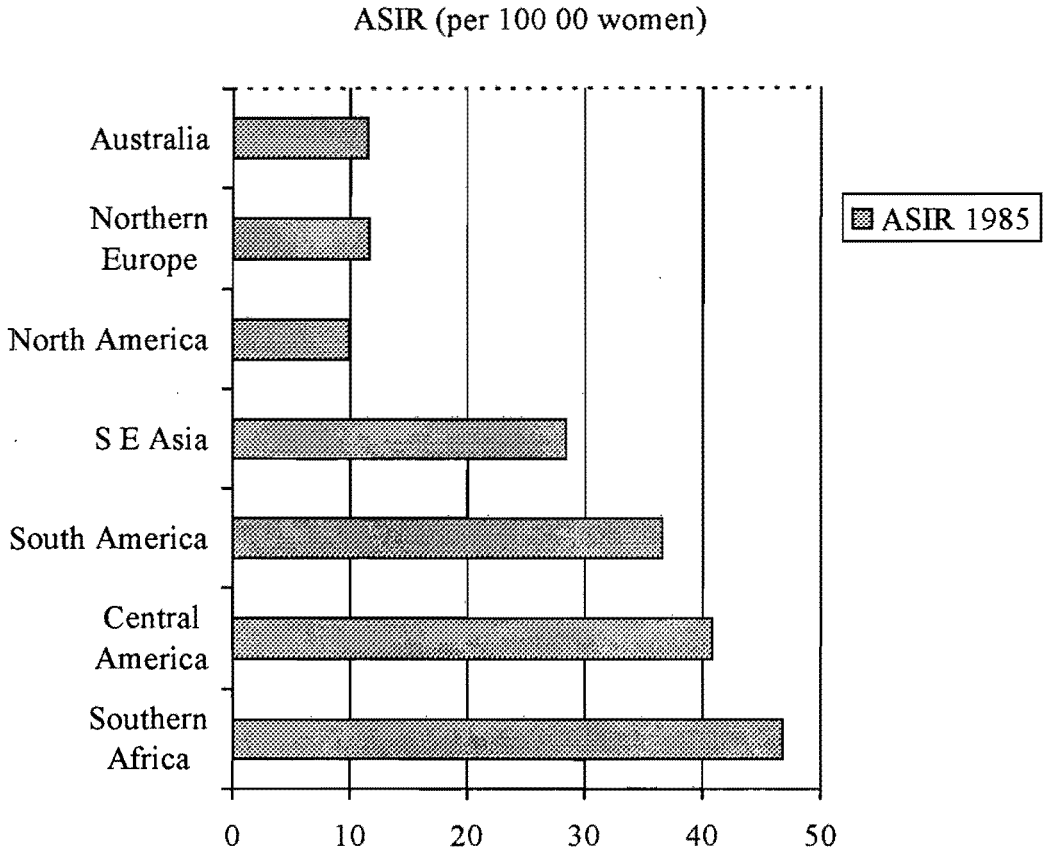


Figure 2: Age Standardised Incidence Rates of Cervical Cancer (Parkin 1985)[3]

### 1.1.1 Biological basis for cervical cancer screening: The Cervical Transformation Zone

The cervical epithelium is derived from two embryologically distinct sources. The portio vaginalis of the cervix is covered by non-keratinised stratified squamous epithelium, similar to the vaginal epithelium. Mucus-secreting columnar cells of the same embryological derivation as the uterine endometrium cover the endocervical canal. At birth there is an abrupt junction between the original squamous epithelium and the columnar epithelium of the endocervix, known as the original squamo-columnar junction (OSCJ). In the majority of cases, this junction is on the ectocervix, but may be on the vagina, particularly in di-ethylstilbestrol (DES) exposed women. At about one year of age, the cervix begins to elongate resulting in the migration of the squamocolumnar junction towards the external os. At the time of menarche or during pregnancy, both the uterus and cervix enlarge. Enlargement of the cervix is accompanied by alterations in its shape, resulting in greater eversion of the endocervical columnar epithelium towards the vagina.

Over time, the columnar epithelium exposed on the portio vaginalis of the cervix is remodelled and replaced by metaplastic squamous epithelium. As this occurs, the original squamocolumnar junction (OSCJ) moves towards the endocervical os or endocervical canal, creating a new SCJ. The area between the original and the new SCJs is called the transformation zone (TZ) and is characterised histologically by metaplastic epithelium.

The concept of the TZ is central to the understanding of the pathogenesis of squamous cell cancer of the cervix and its precursors, because virtually all cervical squamous neoplasia originates at the new SCJ and coincides with the distribution of the TZ. In addition, in reproductive life, the TZ is located on the exposed portion of the cervix and is amenable to cytological and histological sampling and colposcopic examination. While the TZ is difficult to visualise with the naked eye, its localisation is greatly enhanced by the application of 5% acetic acid and the use of the colposcope.

### 1.1.2 Natural history of Cervical Cancer Precursors

It has long been recognised that cervical cancer develops from histologically well-characterised precursors. The first evidence suggesting the existence of precursor lesions for invasive squamous carcinoma of the cervix were observations made in the late 1800s that non-invasive epithelial abnormalities frequently existed adjacent to invasive lesions. Broders appears to be the first to propose the term carcinoma -in -situ (CIS) to describe these intraepithelial abnormalities[7].

Subsequently a number of case-controlled studies demonstrated that a significant proportion of women with CIS, who were untreated, developed cervical cancer. Petersen[8] followed 127 women with biopsy-confirmed high-grade preinvasive lesions of the cervix (called then Epithelial Hyperplasia with Nuclear Abnormalities) for a minimum of 3 years. He found that overt cervical cancer developed in 4% of

women at the end of one year, 11% at the end of 3 years, 22% at the end of 5 years and in 33% at the end of 9 years of follow up. Kottmeier found that 25 of 34 women with CIS who were followed for 20 years or more without treatment developed invasive cancer [9].

In a study by Koss et al[10], CIS lesions were confirmed histologically by biopsy and then followed using cytology alone. Six percent of the women (4/67) developed invasive cancer between 16 and 54 months after entering the study and 5/67 women developed possible invasion. Hall and Walton[11] reported a 29% progression rate of severe dysplasia to carcinoma in situ or invasive cancer over a 1 – 14 year period.

In the 1950s it became apparent that there were another large group of cervical lesions that had some of the characteristics of CIS, but to a lesser degree. Reagan[12] first introduced the term dysplasia to describe these lesions. Dysplasia referred to abnormalities that included a cytological and histological spectrum of lesions intermediate between CIS and normal epithelium. The WHO (World Health Organisation) adopted this terminology as mild, moderate, severe dysplasia and CIS for cytological and histopathological classification of cervical cancer precursors.

The natural history of dysplasia was studied extensively in the 1950s and 1960s. Population based screening programmes of previously unscreened populations showed that women with mild dysplasia were younger than women with moderate dysplasia who in turn were younger than women with severe dysplasia and CIS[13]. This age distribution suggested that mild dysplasia progressed over years to higher grades of dysplasia and finally to CIS. CIS was considered very high risk for progression to invasive cancer and was aggressively treated with cone biopsy or hysterectomy.

### 1.1.3 Development of CIN Terminology

In the 1960s Richart introduced the CIN classification[14]. Laboratory- based studies showed that the differences between the different grades of dysplasia were quantitative as well as qualitative. On the basis of these studies, as well as long-term clinical follow up studies, Richart suggested that dysplasia and CIS constituted a histological continuum rather than a series of discrete entities and introduced the term 'Cervical Intraepithelial Neoplasia', known as CIN. In the original CIN terminology CIN1 corresponded to mild dysplasia, CIN 2 to moderate dysplasia and CIN3 to severe dysplasia and CIS.

The concept of CIN was strongly influenced by the results of a long-term prospective follow up study of 557 women who had had three previous dysplastic smears[15]. After entry, the women were prospectively followed for an average of 36 months using cytology and colposcopy without cervical biopsy. During follow up only 6% of the lesions spontaneously regressed and the remainder either persisted or progressed to higher grades of dysplasia or cancer.

### 1.1.4 The Bethesda Classification of Cervical Cytology

As outlined above, preinvasive lesions of the cervix have been classified using a variety of different terminologies over time. To standardise the 'terminology chaos' that existed because of these various definitions, a new classification of cytological reporting, called the Bethesda system, was devised in 1988[16]. The Bethesda system combines clinically similar intraepithelial diagnoses into broad categories, specifically low-grade Squamous Intraepithelial Lesions (LSIL), representing the changes of koiliocytic cytological aytpia and CIN1, and high-grade SIL (HSIL), representing the changes of CIN 2 and 3. The new classification was designed for use in cytological screening and it remains technically more correct to use the more detailed CIN scale when discussing histopathology. However, as the behaviour of low-grade lesions differs from high-grade lesions, this study will use the terms LSIL and HSIL for both cytological and histological diagnosis.

LSIL is common and represents the usually benign cytopathological signs of HPV infection. In contrast, HSIL is rare and represents a truly premalignant condition. Although LSIL can be viewed as an epidemiological exposure or risk factor for cervical cancer, HSIL can be viewed as more closely linked to the cancer outcome.

While this conceptual distinction is clinically useful, it is not perfect. There exists a continuum of changes encompassing LSIL and HSIL without a clear endpoint. At the microscopic level, for example, the characteristic cells of LSIL are abnormal but terminally differentiated. The atypical cells progress to the surface, produce keratins, die and ex-foliate as would normal cells. The gradient from LSIL to HSIL is characterised by increasing nuclear atypia and failure of cellular differentiation in progressively more superficial levels of epithelium, with CIN3 representing full-thickness replacement of the epithelium with undifferentiated, atypical cells.

## 1.2 Aetiology of Cervical Cancer: The Role of Human Papillomavirus Infection of the Cervix

For over 100 years it has been recognised that, the epidemiology of squamous cancer of the cervix has many of the characteristics of sexually transmitted diseases[17]. Sexual factors such as early age of first intercourse, early age of first pregnancy, multiple partners or a partner who has multiple partners and a history of sexually transmitted diseases are known to increase a woman's risk of developing cervical cancer[18-20]. This strong association with sexually transmitted diseases stimulated the search for an infectious agent as the cause of cervical cancer and its precursors.

Initial attempts to identify an infectious agent as the aetiological agent for cervical cancer focussed on known genital pathogens as the causative agents, particularly infections by bacteria (T.pallidum, N.gonococcus), protozoa (T.vaginalis) and Chlamydia. None of these attempts produced conclusive results. In the late 1960s genital infection by herpes simplex virus (HSV) type-2 was considered a promising candidate, when sero-epidemiological studies revealed higher antibody titres against HSV-2 antigens in cervical cancer patients compared to age-matched controls[21]. Subsequent studies however, failed to support an involvement of HSV-2 infections in cervical cancer or preinvasive lesions of the cervix[22].

In the 1970s zur Hausen[23, 24] first suggested a role for the Papillomaviruses in the pathogenesis of genital tract cancer. There is now a substantial body of epidemiological, clinical and molecular evidence that persistent infection of the cervix with certain types of Human Papillomavirus (HPV) is the central cause of cervical cancer and its precursors[25, 26].

### 1.2.1 Human Papillomaviruses

HPVs are non-enveloped, double stranded icosahedral DNA viruses that are classified into various 'types'. HPV types are defined by genomic analyses and therefore represent genotypes. The different types of HPVs are characterised according to DNA sequence homology. The types are numbered sequentially when they are characterised. Each type has its own tissue predilection and disease spectrum. At present, novel HPV genomes are described as a new HPV type if the nucleotide sequences of the E6, E7 and L1 genes (i.e. about one-third of the genome) differ by more than 10% from those of any previously described HPV types[27].

To date approximately 100 genotypes of HPV have been identified, and 30 types are believed to infect the genital tract. HPVs infect epithelial cells and are restricted to this target population of cells[28]. Papillomaviruses are widely distributed throughout mammals and are highly species specific[29]. Different types of HPV have considerable specificity with regard to the epithelium they infect and the morphology of the lesions they produce. For example, HPV types 1 and 4 are associated with deep plantar warts, whereas HPV 2 is associated with common warts in the general population. HPV types 6

and 11 cause genital warts as well as laryngeal polyps. The following HPV types typically infect the cervical epithelium: 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42- 45, 51 – 58, 59, 66 and 68.

### 1.2.2 Transmission of Genital HPV Infection

Observational studies suggest that genital HPV infection is transmitted primarily through contact with infected cervical, vaginal, vulvar, penile or anal epithelium, indicating that genital HPV infection is sexually transmitted[30]. In studies of sexually inexperienced young women, HPV DNA and antibodies to genital types of HPV are rarely detected[31].

In addition, there appears to be a positive association between increasing numbers of recent partners and increasing prevalence of genital HPV infection[32]. In a cohort study of 18 – 20 year old female university students with a low-risk profile for sexually transmitted diseases, the overall prevalence of genital HPV infection as determined by PCR-analysis was 35%[32]. The prevalence at enrolment among the 183 sexually experienced women ranged from 17% of those reporting only one partner to 83% of those reporting more than five partners[32].

### 1.2.3 Natural History of HPV Infection and Evidence that HPV Infection causes most Cervical Neoplasia

There is a limited understanding of the natural history of genital HPV infections, mainly because the virus cannot be grown in tissue culture and is therefore particularly difficult to study. The initial site of HPV infection is assumed to be the germinal cells of the basal layer of the epithelium, with the virus gaining entry via minor epithelial injuries. The protein products of the E6 and E7 Open Reading Frames of the HPV genome appear to be principally responsible for the HPV neoplastic effects. The E6 protein binds to and inactivates p53 tumour suppressor protein and the E7 protein binds to and inactivates the retinoblastoma tumour suppressor protein, disrupting cellular regulation.

The HPV genome is maintained in the cell nucleus and is usually episomal. In invasive cancers however, HPV DNA integration into the host genome is found in the majority of the cases[33]. The relative expression of E6 and E7 proteins is maintained once the HPV DNA is integrated into the host genome. At the molecular level, human cancers result from an accumulation of genetic mutations. In the molecular pathogenesis of cervical cancer, persistent infection with an oncogenic HPV expressing E6 and E7 proteins, is the initiating event. Thereafter, somatic genetic mutations occur that may be involved in cervical cancer development.

Only a small percentage of individuals infected with HPV develop cervical cancer[34, 35] and the usual pathogenesis of HPV infections is benign. Cervical HPV DNA tends to disappear within months to a few years of detection, possibly due to suppression of the virus by the host and the cessation of detectable viral shedding[36, 37].

Some HPV infections are however, persistent. HPV DNA persistence may be an early and important step in the development of genital lesions such as warts and neoplasia. Hildesheim et al[36] observed that women infected with HPV types known to be associated with cervical cancer were more likely to have persistent infection than those infected with other HPV types. In addition, women developing CIN under observation were more likely than controls to have persistent rather than transient DNA detection.

The role of persistent HPV infection with high-risk types of HPV in the development of progressive preinvasive disease is supported by a study performed by Remmink et al[37]. This group screened a total of 342 women with abnormal smears every 3 – 4 months with cytology, colposcopy and HPV DNA testing using PCR. Nineteen women showed progressive preinvasive disease and all were continuously HPV DNA positive from the beginning of the study and all had histologically confirmed CIN 3. No cases of progressive disease were noted in HPV DNA negative patients or those infected with low risk types of HPV DNA. Using life-table analysis, the cumulative rate of progressive, histologically-verified CIN disease was 17% after 36 months.

### 1.2.4 The Causal Relationship between HPV Infection and Cervical Cancer

The epidemiological association between HPV infection and cervical cancer fulfils all the established epidemiological criteria for causality[38]. These criteria include strength and consistency of the epidemiological association, time sequence, specificity of the association and coherence with existing biological and epidemiological evidence. The association between HPV infection and cervical cancer is remarkably strong and consistent, with virtually no negative studies[39].

In case series worldwide, most cervical cancers have been found to contain HPV of the same 10 – 15 types. Metastases contain the types of HPV found in the corresponding primary tumours. The most definitive study of invasive cervical cancer included 1050 cervical cancers from 20 countries, tested for all known HPV types by PCR: 93% of the cases had detectable HPV DNA, with HPV type 16 being the most commonly detected type[40].

Regarding a logical time sequence, HPV infection (as measured by DNA) tends to precede and predict incidence of cervical neoplasia. Early results from large prospective studies of cytologically normal women show substantially elevated relative and absolute risks of incident SIL, including HSIL, within a few years of viral DNA detection. Cancer-associated HPV types are associated with a higher risk of development of cytologically evident lesions than are other HPV types. In addition, follow up studies of women with LSIL have found that cancer-associated HPV types predict an elevated risk of progression to HSIL.

The animal and experimental evidence for HPV carcinogenicity are strong, satisfying the causal criterion of 'coherence'. The potential for malignant transformation of papillomavirus-induced lesions

has long been recognised. For instance, in the rare genetic disorder epidermodysplasia verruciformis, patients develop multiple HPV-induced cutaneous warts that are prone to squamous cell carcinomas, especially on sun-exposed areas of the skin[41].

### 1.2.5 HPV Infection of the Cervix over Time and Among Women with Normal and Abnormal Cervical Smears

From a variety of studies the following factors emerge. Most individuals with genital HPV infection do not develop signs or symptoms that are brought to the attention of a clinician. It is however likely that many infections cause microscopic intraepithelial lesions that are never detected [32] .In addition, within a few years of the initial infection, most individuals have no molecular, microscopic or clinical evidence of the initial infection [42].

Polymerase chain reaction (PCR)-based methods of HPV DNA detection have revealed that the prevalence of HPV infection in cytologically normal women ranges from 3.5 – 53%. However, Evander et al[42] showed that over a 2-year period the prevalence of HPV DNA decreased significantly in their cohort of 276 mostly university female students. The steepest drop in prevalence was in the women who were 25 years old at enrolment. In Holland, HPV DNA was analysed by PCR in a cohort of women participating in the screening programme. A gradual decrease in HPV prevalence with age was found, from 14% in women up to 30 years, to 5% of women over 30 years[43].

Koutsky et al[44] studied a cohort of 241 cytologically normal women who had no past history of CIN and had been recruited from a sexually transmitted diseases clinic in Seattle, USA. The population was followed every four months by repeated HPV testing and cytological and colposcopic examinations for an average of 25 months. Based on survival analysis, the cumulative incidence of biopsy-confirmed high-grade CIN among HPV positive women was 28% at two years, compared with 3% among HPV negative women. Most of the incident high-grade CIN occurred within the first two years of follow up.

Nobbenhuis et al[45] monitored 353 women referred to gynaecologists with CIN 1, 2 and 3 with cytology, colposcopy and testing for high-risk HPV DNA with PCR. The median follow-up time was 33 months. Thirty-three women reached clinical progression and all 33 had persistent infection with high-risk HPV. The cumulative 6-year incidence of clinical progression among these women was 40% (95% CI 21 – 59). In women with end histology of CIN 3, 95% of 103 women had persistent infection with high-risk HPV from baseline. Clinical progression of CIN was not seen in the absence of high-risk HPV infection of the cervix. In addition, among women with CIN 1 or 2 at baseline, a second test for HPV at 6 months predicted end histology of CIN 3 better than a second cervical smear. They concluded from their data that persistent infection with high-risk types of HPV is necessary for the development and maintenance of CIN 3.

### 1.2.6 Summary of the Role of HPV Infection of the Cervix in the Pathogenesis of Cervical Cancer

The earliest known step in cervical cancer is the transmission of HPV infection. Although sexual transmission is the most important route of transmission of HPV, fomite transmission of HPV to the cervix appears theoretically possible based on findings of HPV DNA on underclothes and gynaecologic equipment[46]. Vertical transmission of genital types of HPV is certainly possible, although the frequency is unknown[47, 48]. The cumulative lifetime probability of acquiring a cervical infection with at least one type of HPV is extremely high for sexually active individuals. The HPV prevalence of a given population depends most strongly on the age and sexual practices of the population. Young, sexually active women have the highest HPV prevalence[49, 50].

The current model of cervical cancer pathogenesis can be summarised in the following way. The central event is infection of the cervix with HPV. Infection of the cervix in most women is transient, but in some women infection is persistent. Persistent infection of the cervix with high-risk types of HPV results in morphological alteration of the epithelium beginning with koiliocytosis, followed by dysplastic change, representing precursors of cervical cancer, and ultimately the development of cervical cancer in some women.

## 1.3 Do Mass Cervical Cancer Screening Programmes Prevent Cervical Cancer?

### 1.3.1 Cervical Screening Programmes in Developed Countries

There have been no randomised trials to evaluate the impact of cervical cancer screening on cervical cancer incidence and mortality and all data on the effect of screening has come from cohort and case-controlled studies. However, the marked reduction in the incidence of and mortality from cervical cancer before and after the introduction of screening programmes in a variety of developed countries, has been interpreted as strong non-experimental support for organised cervical cancer screening programmes.

The International Agency for Research on Cancer (IARC) conducted a comprehensive analysis of data from several of the largest screening programmes in the world in 1986 and showed that well-organised screening programmes were effective in reducing the incidence of and mortality from cervical cancer[51]. In the Nordic countries, following the introduction of nationwide screening in the 1960s, cumulative mortality rates of cervical cancer have shown a falling trend. The greatest fall was in Iceland (84% from 1965 to 1982) where the screening interval was the shortest and the target age range the widest. The smallest reduction in cumulative mortality (11%) was in Norway where only 5% of the population had been part of organised screening programmes[52]. The falls in Finland, Sweden and Denmark were 50%, 34% and 27% respectively. The highest reduction in cervical cancer incidence was in the 30 – 49 age groups where the focus of screening was the most intense.

In addition, the association between mortality trends and the extent of coverage of the population by organised screening was most pronounced when the proportional reductions in the age-specific rates were related to the target ages of the screening programmes. The age-specific trends indicated that the *target age range* of a screening programme was a more important determinant of risk-reduction than the *frequency* of screening within the defined age range. This finding was in agreement with the estimates of the IARC working group, that for inter-screen intervals of up to 5 years, the protective effect of organised screening was high throughout the targeted age group (over 80%) [53]. It is apparent therefore that the extent to which screening programmes have succeeded or failed to decrease incidence of and mortality from cervical cancer is largely reflected in 1] the extent of coverage of the population at risk by screening, 2] the target age of women screened and 3] the reliability of cytology services.

The contrast between Finland, which had an organised screening programme and Norway, where an equivalent number of smears were performed opportunistically, indicated another important aspect of screening. Even though the difference in the total number of smears taken in the two countries was not great, the reduction in mortality was substantial for all ages in Finland, whereas in Norway, only women aged 30 – 49 years showed a fall in mortality rates: even for that age group, the fall was only half that in Finland. These data suggest that spontaneous or opportunistic screening fail to reach the most at risk women in the population, that is, middle-aged and older women of high relative risk and therefore has far less of an impact on the incidence of and mortality from cervical cancer. Other

reasons for the failure of opportunistic screening to reduce cervical cancer mortality include sub-optimal follow-up and management of women with abnormal smears and the lack of a co-ordinated campaign of informing and educating women about cervical cancer prevention. This results in women at high risk of disease being excluded from screening[54].

Further evidence of the impact of screening is found in the Canadian experience where the incidence of cervical cancer dropped from 28.4 to 6.9 and mortality from 11.4 to 3.3 per 100 000 women during a 20 year screening programme in British Columbia[55].

Mortality from cervical cancer in the United Kingdom fell by 30% after the introduction of screening in the 1960s, however some of this decrease in mortality was attributed to falling rates in older women and could have been a cohort effect unrelated to screening[56]. The need for an effectively managed national programme in the UK was realised by the mid 1980s, which led to the introduction of a computerised call and recall system for women aged between 20 and 64 years. The invitation- based system, together with target payments for general practitioners, improved population coverage from 40 – 60% in 1989, to 80% in 1992 and to 83% in 1993. In an audit of this programme in 24 self-selected districts in the UK by Sasieni et al[56], it was estimated that the number of cases of cervical cancer in the participating districts would have been 57% (95% CI: 28 – 85%) greater had there had been no screening. Further they estimated that screening prevented between 1100 and 3900 cases of invasive cervical cancer in the UK.

### 1.3.2 Cervical Cancer Screening Programmes in Developing Countries

In many developing countries screening is either opportunistic, sporadic or does not occur at all. In 1986 the WHO estimated that while approximately 40 – 50% of women in developed countries had been screened in the past 5 years, only 5% of women in developing countries had been screened[57]. In addition, most screening activity in developing countries was limited to women attending primary health care, antenatal and family planning clinics in urban areas, with no organised efforts to ensure that high-risk women attended for screening, treatment and follow up[57]. While there is a paucity of data on screening programmes and incidence of and mortality from cervical cancer in many developing countries, there have been a number of attempts to establish screening programmes. Some of these programmes have met with marginal success, many however, have failed to either become established or to make an impact on cervical cancer incidence and mortality.

### 1.3.3 Screening in South Africa, with Particular Reference to Khayelitsha

There has never been a national, organised cervical cancer screening policy in South Africa and cervical screening has been performed opportunistically, largely in family planning and antenatal clinics[58]. An example of the typical screening activity encountered in South Africa is illustrated in a study performed by Baillie in the Western Cape in 1994[59]. Baillie collected data on cervical smears

analysed by the University of Cape Town Cytology laboratory over a five year period and showed that the highest proportion of smears were performed in maternity obstetric units on pregnant women under the age of 30 years. However, the highest proportion of smears showing CIN 3 was found among women aged 30 – 39 years who were screened in local clinics, and the highest proportion of smears showing malignancy were found among women over the age of 50 years, who were screened opportunistically in day hospitals. The peak incidence of screening overall occurred in the 20 – 29 year age groups, and the screening incidence for women over 40 years or more was one third to one tenth of the peak screening incidence.

The screening activity in Khayelitsha (the area in which this study was performed), follows a similar pattern to that reported by Baillie[59]. Since 1987, cervical smears in Khayelitsha have been obtained from women presenting for health care at three primary health care clinics, three family planning clinics and one Maternity Obstetric Unit (MOU). All cervical smears taken in Khayelitsha were

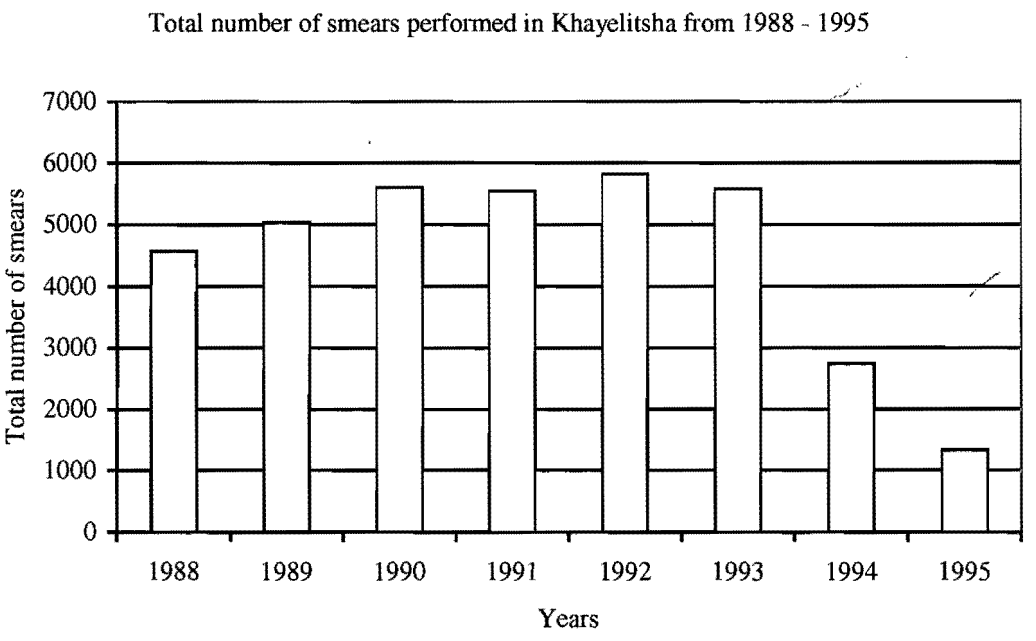


Figure 3: Total number of smears taken per year in the Khayelitsha area from 1988 – 1995

evaluated at the UCT Cytopathology laboratory. The total number of smears taken per year in Khayelitsha from 1988 – 1995 are illustrated in Figure 3 (data extracted by the author from the laboratory computerised database established in 1987).

From 1988 to 1993 over 5 000 cervical smears per year were taken in Khayelitsha, with a population estimated at between 350 and 500 000 people. After 1994, the number of smears recorded in the laboratory declined to 2 743 and in 1995, to 1 332. This was due to the adoption of a new policy by the Provincial Administration of the Western Cape 1994. This policy, designed in principle to focus screening on older women, stated that 3 free cervical smears would be offered to asymptomatic women

every decade beginning at age 30. This policy resulted in a marked reduction in the total number of smears performed in Khayelitsha, largely because screening of pregnant women and women under the age of 30 years virtually ceased.

The age distribution of the smears taken in Khayelitsha from 1988 – 1995 is shown in Figure 4. The majority of smears were performed in young women: 11.1% were aged 15 – 19 years, 61.1% were aged 20 – 29 years, 16.8% were aged 30 – 34 years and 11.0% were over the age of 35 years. After the change in policy in 1994, while the total number of smears taken decreased substantially, the proportion of smears taken in women over the age of 35 increased marginally from 11 to 14%.

There are no reliable data on the extent of coverage of women in Khayelitsha, or on the impact of

Age Distribution of Cervical Smears taken in Khayelitsha 1988 – 1995

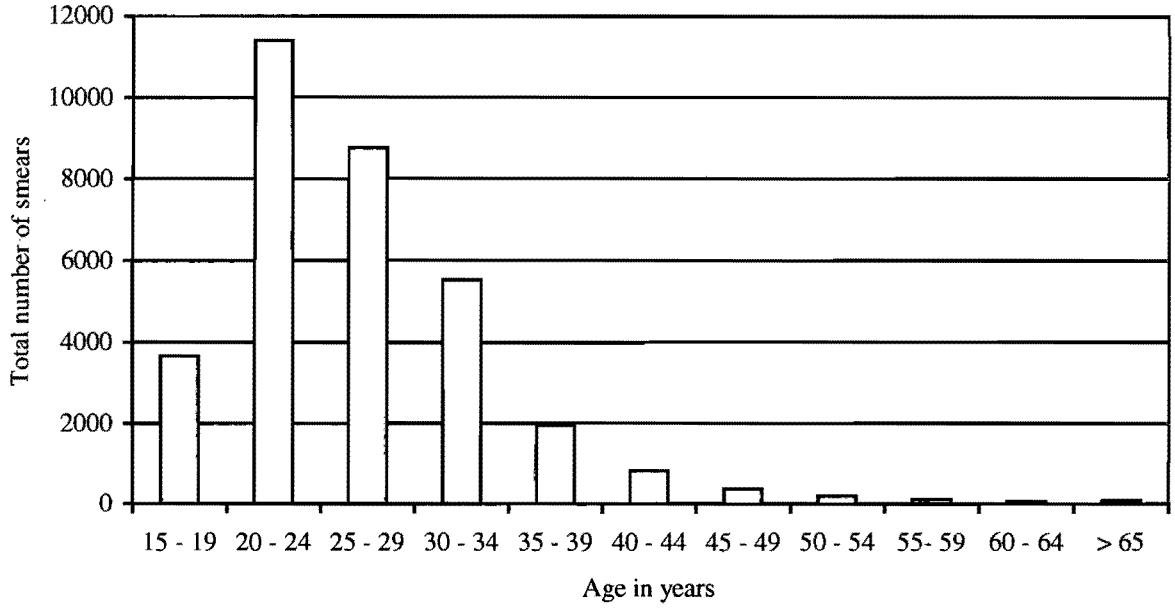


Figure 4: Age Distribution of Cervical Smears taken in Khayelitsha 1988 – 1995

screening on the incidence of cervical cancer in the area. This is partly due to very mobile nature of the population of Khayelitsha, where many women move between their rural homes and the city. However, Megevand et al[60], recruited 5045 women living in Khayelitsha to their study and overall, 5.9% of the women reported that they had previously been screened. In a study by Cooper et al[61], which was a population-based survey of the health status of women in Khayelitsha performed in 1989, 44% of women had had a Pap smear but less than 50% of the women screened knew the results of their Pap smears or had attended for follow-up.

Cronje et al[62] reported a similar pattern of screening in the Orange Free State (OFS), another province in South Africa. Just over 36% of all smears performed in the province were in women aged 25 – 34 years and 29% of smears were performed in women aged 15 – 24 years. By contrast 19% of smears were in women aged 35 – 44 years and only 16% were in women 45 years and older. While white women constituted 21% of the population of the OFS, (a group considered a relatively low-risk for cervical cancer due to their higher socio-economic status and greater access to screening), 53% of the women screened were white. In comparison, 76% of the population of the OFS were black women and only 43% of smears were performed in this group of women. Further, the vast majority of the smears (60%) were performed in the Bloemfontein, the capital of the OFS, with less than 10% of smears performed in rural areas where the majority of black women lived.

In another study conducted in outside Pretoria, South Africa, Heystek et al[63] offered cervical smears to and assessed knowledge of screening among 1095 attending a local clinic for non-gynaecological complaints. Only 2% of the women interviewed had any knowledge about Pap smears and the incidence of preinvasive disease of the cervix was 54/ 1000 women, which is relatively high.

### 1.3.4 An Experience in South Africa: 'Project Screen Soweto'

Another example of screening activity in South Africa is illustrated by a study in Soweto, South Africa, called 'Project Screen Soweto'[64]. In 1980, at Baragwanath Hospital, a large tertiary institution serving the inhabitants of Soweto (an African township outside Johannesburg, South Africa), an approximately 50% increase in the admissions for cervical cancer, from 150 cases in 1970 to 236 cases in 1980 was documented. In addition, in excess of 70% of women with abnormal cervical smears had not been followed up. In recognition of the poor quality of the existing opportunistic screening programme and the apparently rising incidence of cervical cancer, Project Screen Soweto (PSS) was initiated in 1986[64].

Prior to the establishment of Project Screen Soweto, a few basic goals were accomplished. Firstly, laboratory capacity for screening had been increased from 30 000 to 90 000 smears per annum. Pilot studies had identified the prime screening target as an existing and available pool of patients through the network of primary health care clinics. Laboratory-based follow-up services had been established and streamlined, and channels for communication between patients, clinicians and the laboratory had been created. The only unresolved problem at the time was the development of an accessible public education programme. This problem arose because of lack of resources but also due to fears that too vigorous and too early a public education programme would flood an untested screening network, swamping primary health care clinics, the laboratory, colposcopy clinics and the hospital wards. A decision was thus made to launch Project Screen Soweto prior to instituting any public health educational programme.

During the planning phase of the project in 1982, 32 365 smears were taken. After the launch of the project however, the number of smears taken decreased to 24 251 in 1983 and to 26 216 in 1984. In

addition, there was a rapid decline in the diagnosis of both invasive and preinvasive cervical cancer, the opposite of what should occur in a screening programme conducted on a previously unscreened population. Unfortunately the project failed, despite following the published suggestions of Boyes et al based on their experience of the very successful British Columbia cervical cancer screening programme. The failure of the PSS was acknowledged 5 years after the commencement of the project. It was calculated however, that had available laboratory facilities been utilised to full capacity during that 5-year period, in excess of 300 000 women in Soweto would have received at least one smear, which should have yielded 6000 preinvasive or invasive cancers.

The reasons for the failure of PSS are complex. An important cause of the failure of the programme appeared to be at the level of the administration of primary health care services, where the cervical smears should have been taken, but where the taking of smears was given a very low priority. Although not documented in the report of the PSS, it is likely that competing health needs of the largely poor community contributed to cervical cancer prevention efforts receiving low priority status. In addition, the failure to establish an effective public health educational programme resulted in women remaining ignorant about cervical cancer prevention and no consumer demand for screening was created.

Allocation of financial resources may also have played an important role and the long-term savings created by preventing cervical cancer may not have been appreciated. For instance, with the cervical cancer incidence rates prevailing in 1984 in South Africa, it was estimated that a saving of R204 000 would be obtained for every 1000 women screened, with a very favourable cost-benefit ratio of 0.72[65].

### 1.3.5 Preliminary Results from a South African Study Designed to Formulate an Affordable Population Screening Programme

In a more recent attempt to develop a national screening programme, a nationwide study in South Africa was started in nine provinces in 1997, funded by the Independent Development Trust. The intention was to screen 100 000 women in different regions of South Africa in order to determine the prevalence of cytological abnormalities in the population of South African women. Further aims of the study were to collect information about the capacity of the health services to provide screening services and whether women received results and appropriate action was taken where an abnormality was detected.

Despite best efforts, only 20 277 Pap smears were taken throughout the country, far lower than the intended 100 000 smears. Overall, 80% of women had never been screened and 92% had not had a Pap smear in the last 5 years. Of particular concern, was that only 14.9% of women with HSIL Paps and 13.4% of the women with invasive Paps were recalled for further assessment, although just over 90% of the women screened received their results. This suggests that the interpretation of the smear results by the health personnel, who were responsible for recalling women with abnormal smears, was

particularly poor (Personal communication, Professor Basil Bloch, Principal Investigator of the study and National President of the Cancer Association of South Africa).

### 1.3.6 Screening Programmes in Other Developing Countries

In India, some attempts to establish screening programmes through hospital or clinic outpatient services and the use of mobile cancer detection clinics, have shown a reduction in cervical cancer incidence. Luthra et al[66] reported on a study in which 38 707 women were initially screened over a three-year period from 1960 to 1963 and repeat screening was performed on 26 110 women from the same population between 1966 and 1970. The incidence rates of cervical cancer were reduced in the two time periods from 1.7% to 0.63% and the corresponding rates for dysplasia increased from 2.4% to 6.28%.

In Costa Rica, nationwide cytology services have been available since 1970 for women aged 15 years and above receiving obstetric and gynaecological care. In 1987 a total of 242 219 smears had been taken (equivalent to 287.3/1000 women above 15 years of age). However, most of the women over 55 years of age had never been screened. The overall crude and age-adjusted mortality rates from cervical cancer had not decreased in the last two decades despite the large number of smears performed[67].

In Cuba, a cervical cancer screening programme was initiated in 1967 for women aged 20 years and older with a screening interval of 2 years. Approximately 60% of women aged 20 – 60 years have been screened at least once. Coverage has been reported as 74% among women aged 20 – 25, but only 20% for women aged 50 years and older. Although no reduction in the incidence and mortality from cervical cancer in Cuba has been observed in the last three decades, the proportion of stage I cancer has steadily increased from 26% in 1982 to 50% in 1988 with a proportional decrease in stages 2 and 3 cervical cancers[68].

In Cali, Colombia, cytological screening has been offered to all sexually active women through antenatal clinics since the late 1960s and a decrease in the incidence of cervical cancer has been observed [69]. The age-adjusted rates of cervical cancer were 52.9, 48.2 and 42.2 (per 100 000 women) during 1972 – 1976, 1977- 1981 and 1982 -1986 respectively. The same group showed in a case-controlled study that the relative risk for invasive cervical cancer was 9.9 among women with no history of screening, compared to apparently healthy women with a history of screening.

There has been a marked decline in cervical cancer incidence in all age groups in Shanghai, China and Hong Kong and this has been attributed to cervical cytology programmes initiated in the early 1970s[70]. In Jingan, in a rural community in China, 95% of the eligible population attended for screening at least once over a 12-year period between 1974 and 1985[71]. In a case-controlled study reported from this region, the relative risk of cervical cancer was found to be 0.33 in women who had three or more smears. In contrast, a mass screening programme provided by the Cancer Society of Taiwan between

1974 and 1984, through some 700 gynaecological clinics throughout the country, managed to screen only 5.3% of the eligible women with no impact on cervical cancer incidence[72]. In another case-controlled study conducted among Thai women in Bangkok, a consistently decreasing risk of invasive cancer was observed with increasing frequency of Pap smears. Compared to unscreened women, risk of cervical cancer was reduced by approximately 75% when one Pap smear per year was performed[73].

In Mexico, where nationwide cervical cancer screening was implemented in 1974, the mortality rate of cervical cancer has remained unchanged. In a study by Lazcano-Ponce et al[74], it was found that coverage of women age 35 -64 years was between 10 - 15%, the age group at highest risk for cervical cancer. Low utilisation of the screening programme by women at high risk was one of the main reasons that the screening programme had been ineffective. In another Mexican study, the quality of cervical smears was found poor. For example, in a random sample study of the quality of cytological smears at the Hospital General de Mexico, 64.1% of samples were of low quality and the correlation between diagnostic error and low quality of the smear was very high at 0.87 ($p<0.005$) [75].

These data on screening programmes in developing countries indicate, that establishing screening programmes is complex and often not possible despite best efforts. Clearly, besides considerable human, technical and financial resources, a series of parallel processes need to be instituted for screening programmes to have the desired effect of reducing incidence of and mortality from cervical cancer. Most developing country programmes described in the literature tend to be low-volume, based in laboratories with little in-built quality control measures, isolated and opportunistic without any systematic recruitment, follow-up and treatment of women at risk. Coverage tends to be poor, targeting young women who are often screened too frequently.

### 1.3.7 Criteria for Successful Screening Programmes

Experience from countries that have successfully maintained cervical cancer screening programmes indicate that instituting mass cervical screening requires a complex network of parallel factors[76, 77].

The following factors are considered important to ensure the success of cytologically-based screening programmes:

- Adequate field facilities with appropriately trained health personnel to perform quality cervical smears
- Wide coverage of the target population (at least 70%) at regular intervals : a minimum of 3 years between smears and mechanisms for contacting women on an individual basis e.g. through personally addressed letters
- An efficient health infrastructure with functioning management and information systems
- A cytology service with built-in quality assurance, ongoing training of cytotechnicians and regular audit of performance of laboratories

- An accessible and functional referral system for colposcopic assessment of women with abnormal smears
- Adequate diagnosis, treatment and follow up of women with abnormal smears and follow up of women who have been treated for preinvasive disease of the cervix
- Community understanding and acceptability
- Screening programmes should be planned at national level, they should be organised to encompass a call and recall system.
- A cancer registry to monitor the impact of the screening programmes

These factors require a relatively sophisticated health infrastructure. With respect to these requirements, what is the situation in developing countries? The next section will explore some of the barriers to establishing screening programmes in developing countries.

### 1.3.8 Barriers to Cervical Cancer Screening in Developing Countries, with Particular Reference to Sub-Saharan Africa.

There is not a single developing country that has successfully implemented mass, organised cervical cancer screening, yet the greatest burden of cervical cancer is found in developing countries[78]. There are many explanations for the failure to institute mass cervical screening programmes in developing countries and these will be discussed in the following sections.

#### 1.3.8.1 Competing Health Needs

The burden of diseases other than cancers is overwhelming in developing countries. In sub-Saharan Africa in 1995, it was estimated that communicable diseases and maternal or perinatal complications caused approximately 70 % of all deaths in women: the equivalent figure in developed countries was 4.9%. The overall maternal mortality ratio for sub-Saharan Africa was calculated at 650 maternal deaths per 100 000 live births in 1985, which is the highest of all the world's regions (WHO 1985) [79]. By comparison, the MMR in the USA was 10/ 100 000 live births.

An additional health burden that is expected to consume significant resources is that of HIV infection. In 1994, the World Health Organisation[80] estimated that globally more than 16 million adults and 1 million children had been infected with Human Immundeficiency Virus (HIV). By mid 1994, 40% of the estimated cases of disease had occurred in women and this percentage is expected to increase significantly by the year 2000. In Africa it is estimated that among over 10 million cumulative infections, more than 50% have occurred in women.

#### 1.3.8.2 Limited Human and Financial Resources:

In March 1997 a conference on Cervical Cancer Prevention and Control in East and Southern Africa was held in Kenya and reports on the facilities for cervical cancer prevention and treatment were presented by representatives of 14 health ministries[81]. Most countries reported high incidences of cervical cancer coupled with extremely limited facilities for screening or treatment. In Malawi, which established a population based cancer registry in 1988, the incidence of cervical cancer was reported as 47/100 000 women. Yet, the country has one pathologist, one colposcope, no cytotechnicians, no facilities for cervical cancer screening and no facilities for the treatment of cervical cancer.

In Kenya, where there is no cancer registry but the incidence of cervical cancer is believed to be equivalent to Malawi, only 2000 cervical smears per year were recorded in the state health service. There was only one laboratory in the whole country that had facilities to screen cervical smears, but a severe shortage of trained cytotechnicians had made screening virtually impossible. Mozambique had one pathologist, no cytotechnicians and the only facility for cervical screening was located at a large tertiary hospital in Maputo. In Tanzania, with a population of 30 million people, facilities for

opportunistic screening existed in 40 primary health care clinics, but there were no facilities for the training of cytotechnicians and there was no colposcopic service in the state health sector.

By comparison, in South Africa, with a population of 40 million people, there are 217 trained cytotechnicians and 781 specialist gynaecologists and all major centres have adequate facilities for the treatment of cervical cancer and its precursors. Despite these relatively good facilities, there has been no national screening programme and as indicated previously, cervical cancer remains the commonest cancer in women, particularly black women. A national screening programme has only recently been formally adopted (October 1999), which aims to provide three free cervical smears to all asymptomatic women over the age of 30 years. The gap however between policy and implementation remains and the intention is to aim to screen 80% of the population at least once in the next 10 years (Personal communication, Mrs Christelle Kotzenberg, Director Cancer Control Programme, Ministry of Health, South Africa).

The gap between policy and implementation is well illustrated in the Western Cape, South Africa, where the policy of three free smears in a lifetime was introduced in 1994. As shown above the impact of this policy was a dramatic reduction in the number of smears performed without an appreciable increase in smears performed in women over the age of 30 years.

### 1.3.8.3 Poorly Developed Health Care Services

Primary health care facilities where preventative health care such as cervical screening should most likely be located are limited, under -resourced and over-burdened in most developing countries. Only 60% of the population of sub-Saharan Africa have access to modern health care services[82].

In terms of access to health care, the physician: population ratio for the region in 1990 as a whole was 1:23 540, with a range of 1:750 in South African urban areas to 1:72 990 in Rwanda. The regional nursing person: population ratio was 1:3 460 ranging from 1:600 in Zimbabwe to 1:5 470 in Tanzania. However, there was a relatively high ratio of nursing persons to physicians (5:1) for the region as a whole which is a favourable ratio for achieving health worker coverage of the population, compared to having an under-supply of physicians[82]. A contributing factor to limited access to health care is the urban-rural bias, which is extreme in sub-Saharan Africa[82]. While 87% of the region's urban population has access to health services, more than 50% of people in most of its countries live more than 10 kilometres from the nearest primary care centre[82].

### 1.3.8.4 Focus on Curative rather than Preventative Health Care:

It has been estimated that the per capita yearly budget for health care expenditure in most African countries is US$10. These limited resources tend to focus on treating illness once it has occurred rather

than being invested in large preventative health programmes. One exception to this is the HIV campaign in Uganda[83].

### 1.3.8.5 Women are Uninformed and Disempowered

The World Development Report has cited education as an essential component to human health, stating that ' Households with more education enjoy better health, both for adults and for children, [a result that] is strikingly consistent in a great number of studies, despite differences in research methods, time periods, and population samples'[84]. Women in developing countries tend to be poorly educated which has profound ramifications on the total quality of their lives, ranging from health care access, to health seeking behaviour, to the ability to generate income. The poor education of women in developing countries is reflected in the illiteracy rates.

The basic literacy rate for women aged 15 and over has increased over the past few decades to at least 75% in most countries of Latin America, the Caribbean and South Eastern Asia. High rates of illiteracy however, still prevail among women in most of Sub-Saharan and northern Africa, with illiteracy rates of up to 70% reported[85]. In addition, it is estimated that illiteracy rates for rural women are 2 to 3 times higher than those of urban women, and for older women (over the age of 45 years), illiteracy rates are at least twice those of women younger than 25 years[85].

In South Africa, which is one of the most developed countries in Sub-Saharan Africa, large sections of the population also lack adequate education. For instance, 20% of the population over the age of 20 years have had no schooling at all, 7.5% have completed primary school and 33.9% have had some secondary level education. The majority of people with little or no education are black women[86].

Not only are women in developing countries poorly educated but in most societies they have a status subservient to men with less control over family resources, minimal access to money and in general, inferior social power[82]. This combined, with poor education, has resulted in very low awareness about diseases such as cervical cancer and the fact that the disease can be prevented. Thus there is no consumer demand for services such cervical screening.

### 1.3.8.6 War and Civil Strife

In many countries in Africa, to name a few, Angola, Ethiopia, Eritrea, Rwanda, Liberia, Mozambique, Somalia, South Africa and more recently, the Democratic Republic of Congo, civil upheaval and general violence have been the status quo for decades. Some of the important consequences of war include displacement of people, the creation of refugees, disruption of health care services with loss of infrastructure and personnel and the diversion of state money to defence. For instance, it has been estimated that 30% of the trained health personnel in Rwanda were murdered in the 1994 genocide. These factors make the establishment of successful screening programmes particularly difficult.

### 1.3.8.7 Widespread Poverty

As an indication of the widespread poverty in Sub-Saharan countries, 41% of the total population of the region has access to safe water and 26%, access to sanitation; these are the lowest percentages of all the developing country regions[82]. The difference between urban and rural areas is marked: 79% of the region's urban population has access to safe water as opposed to 28% of the rural population.

In South Africa, 33% of the black population have running tap water inside their dwellings (compared to 97% of the white population) and 22% have flush toilets inside their homes (compared to 98% of the white population). In the Western Cape, 18.6% of the economically active people are unemployed with the highest unemployment rate among Africans being 31% compared to 7.7% among the white population[87].

### 1.3.8.8 The Nature of the Screening Test

While the factors described above are probably the most important reasons that cervical screening programmes have not been established in developing countries, the nature of the current screening process is also a contributing factor. For many developing countries, setting up quality national cytologically-based screening programmes is beyond their capacity and resources.

The first barrier to cytologically-based screening programmes is to develop the necessary infrastructure to perform Pap smears, transport the smears to laboratories where the appropriate processing and interpretation of slides can be performed. Thereafter the results need to be communicated to the referring clinic, and to the women who have been screened. This delay in itself is known to be a significant barrier to screening, with large numbers of women not returning for results[60].

Secondly, for cytologically-based screening programmes to be effective, high quality cytology laboratories need to be established. Interpreting cervical smears is considered by many pathologists to be one of the most difficult tasks in pathology and obtaining a high level of proficiency requires several years of training. Maintaining skills requires ongoing continuing medical education, close supervision by trained laboratory managers and an established built-in quality control programme.

Once a woman with an abnormal smear has been identified, she requires referral for colposcopic assessment. Colposcopy, where available, tends to be located in tertiary, urban-based institutions and provided by specialists. This requirement creates problems of access for poor women, both urban and rural, and contributes to the high default rate identified in a number of studies. For example, Megevand et al[60], reported a 67% default rate for the first phase of their study, when women were referred to a tertiary institution for colposcopic assessment of abnormal Pap smears. A default rate of 40% was recorded by the Groote Schuur Hospital Colposcopy clinic in 1998 (unpublished data by the author).

The modern management of preinvasive lesions has been considerably simplified by the introduction of LEEP (Loop Excision Electrosurgical Procedure), also known as LLETZ (Large Loop Excision of the Transformation Zone). This procedure can be performed in an outpatient setting, using local anaesthetic and relatively unsophisticated equipment. The complication rate of LEEP is low and the reported cure rate ranges from 80 - 95%[90]. The introduction of LEEP has made the treatment of preinvasive lesions more accessible to health care workers, potentially removing this procedure from the domain of the super-specialist. However, the correct use of LEEP is dependent on quality colposcopy, to prevent its unnecessary or inappropriate use[91].

For most countries with limited or no screening, there is a concomitant lack of colposcopic services and outpatient methods of treating preinvasive disease. Hence in those countries with limited screening, e.g. Kenya and Tanzania, most women with abnormal smears are subjected to cone biopsy or hysterectomy; both radical and expensive treatments, which would be unsustainable, were a population-screening programme to exist.

### 1.3.9 Screening in Low-Resource Settings

The failure of cytologically-based screening programmes to be developed and sustained in low resource countries has stimulated the search for methods of screening that would overcome the many barriers identified. To screen successfully in low resource settings the following criteria would be optimal:

- Screening, diagnosis and treatment provided on-site, in clinics accessible to the majority of at risk women
- Low cost/ low technology screening test
- Screening test that can lead to immediate treatment of abnormalities with the elimination of colposcopic triage
- Wide coverage of at risk women
- Appropriate educational programme directed towards health workers and women in the population to ensure correct implementation of the screening programme and attendance and compliance with screening programme
- Built-in mechanism for audit of the impact of the screening programme

The next section will explore possible alternatives to cytologically-based screening programmes with these criteria as the basis for developing alternative methods of screening and treating preinvasive lesions of the cervix.

## 1.4 What are the Alternatives to Cytology?

### 1.4.1 Visual Inspection Methods of Screening the Cervix

### 1.4.2 Unaided Visual Inspection of the Cervix as a Screening test: 'Downstaging'

The first attempts to develop alternative methods to cytology for screening in poor countries originated in India, with the introduction of Unaided Visual Inspection (UVI) of the cervix, also known as 'Downstaging'. This involved the naked-eye speculum examination of the appearance of the cervix by health workers trained in the technique. Women with an abnormal cervix were then referred for a second level investigation, which was either cytology (if available) or for gynaecological assessment if no cytology was available[92-97].

The published studies of 'downstaging' indicate a very wide range (6 - 70%) of women examined by visual inspection were found to have an abnormal-looking cervix, depending on the criteria used to define a positive visual inspection. If any abnormal finding (ranging from excessive discharge up to growth/ulcer) was used to define an abnormal cervix, 41 - 70% were women called abnormal. If more limited criteria were used (bleeding on contact with the cervix, bleeding erosions, hypertrophied elongated oedematous cervix, growth or ulcer) 6 - 11% of women had a positive test. All reported studies used cytology and/or biopsy as a reference test to study the performance of unaided visual inspection.

The reported sensitivities and specificities of unaided visual inspection of the cervix for carcinoma-in-situ and/or invasive cervical cancer are shown in Table 1. The sensitivity to detect high-grade disease and cancer varied from 39 to 94% in different populations, using different providers and different criteria to define a positive test. Attempts to increase the specificity by using a high threshold to define a positive test resulted in unacceptably low sensitivity.

The results from the study by Nene et al[97], which was population rather than clinic based, indicate that downstaging, as a screening tool is unlikely to be useful in cervical cancer control. Even with adequate sensitivity large numbers of women would be identified as positive and would require follow up, off - setting the financial savings of avoiding cytological examinations. When the sensitivity was high, the positive predictive value of the test was less than 1%. Many early or microscopic cancers were missed by unaided visual inspection, as early cancers are often not clinically apparent to the naked-eye examination of the cervix. This explains the poor performance of unaided visual inspection for the detection of cancer and even worse performance for the detection of preinvasive lesions.

Wesley et al[96], who evaluated UVI in 2843 married women in Kerala, India, discussed the cost implications of using low technology methods of screening. If UVI was used to preselect women for cytology, the costs saved would be related to not offering Pap smears to women with normal cervices (just over 50% of the screened population). If 45% of women were referred for further evaluation, large numbers of women would require further investigation, significantly burdening the health system.

In addition, the low sensitivity would result in at least one third of the women with high-grade lesions being missed. They suggest that cost-savings with UVI would not be substantial.

It may be that health education and awareness programmes to motivate symptomatic women to seek medical advice, and professional education to sensitise health professionals to the diagnosis of early cancer, may be as helpful (and less costly) as unaided visual inspection. In a study by Jayant et al[98] on the impact of a health education programme on the stage of diagnosis of cervical cancer, a significant shift in the percentage of cervical cancers diagnosed in early stages (FIGO stages I and II) was found. A group of rural women were subjected to an intensive awareness campaign of symptoms of cervical cancer; 38% of identified cancers were early stage in 1988 - 1989 compared to 51% in 1990 - 1992. The study group was compared to a control group who were not part of the awareness campaign and in whom the diagnosis of early stage cancers did not change over the same time period (38% in 1988 - 89 and 34% in 1990 - 1992). The authors suggest that an active awareness programme may achieve a stage-shift in the diagnosis of early cervical cancer relatively quickly and cheaply.

| Author (yr.) | Visual inspection findings | Cytology/biopsy findings | | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| | | Positive | Negative | | |
| Singh et al 1992 | Positive | 149 | 4986 | 63 | 89 |
| | Negative | 89 | 38746 | | |
| Bhargava et al (1993) | Positive | 50 | 125 | 85 | 96 |
| | Negative | 9 | 3424 | | |
| Sujathan et al (1995) | Positive | 68 | 2199 | 89 | 38 |
| | Negative | 5 | 1330 | | |
| | Positive | 60 | 2207 | 94 | 38 |
| | Negative | 4 | 1331 | | |
| Rao et al (1995) | Positive | 2 | 811 | 5 0 | 59 |
| | Negative | 2 | 1170 | | |
| Nene et al (1997) | Positive | 9 | 1111 | 90 | 43 |
| | Negative | 1 | 833 | | |
| | Positive | 6* | 112 | 60 | 94 |
| | Negative | 4* | 1832 | | |
| Wesley et al (1997) | Positive | 17 | 1262 | 74 | 55 |
| | Negative | 6 | 1558 | | |
| | Positive | 9* | 170 | 39 | 94 |
| | Negative | 14* | 2650 | | |

Table 1: Performance of visual inspection in detecting carcinoma-in-situ and/or invasive cervical cancer [* A high threshold was used to define a positive test] [99]

### 1.4.3 Direct Visual Inspection of the Cervix after application of 5% acetic acid

Direct Visual Inspection of the cervix after the application of 5% acetic acid (DVI) was originally investigated as a tool to improve the sensitivity of the Pap smear. DVI involves visualising the cervix with the naked eye after the application of 5% acetic acid and the identification of aceto-white lesions. One of the earliest published studies on DVI[100] showed DVI was an effective tool at identifying abnormal areas of the cervix for further evaluation. DVI identified 98.4% of the colposcopically

detected 'atypical transformation zones', which contained either preinvasive or invasive lesions of the cervix. The authors suggested that DVI was as good as colposcopy in identifying potentially abnormal areas of the cervix, although DVI lacked the ability to discriminate between significant and insignificant lesions. They suggested however, that DVI could be a useful adjunct to cytology for cervical screening.

Van Le et al[101] performed Pap smears on women attending family planning clinics and applied acetic acid to the cervix in order to identify aceto-white lesions. 85 women with normal Pap smears but positive aceto-white lesions were referred for colposcopic assessment. Of these women with normal Pap smears there were 13 cases of CIN detected by the positive acetic acid reaction; 9 women had CIN1 and 4 women had CIN 2.

Frisch et al[102] showed a decrease in the false negative rate of cytological screening by the addition of DVI; 3 cases of HSIL were identified by DVI that were missed by cytology. The positive predictive value of screening was decreased by combining cytology with DVI (compared to using cytology alone) however the negative predictive value increased from 67 (95% CI: 57 - 77) for cytology used alone to 91 (95% CI: 80 -100) when cytology was used with DVI.

### 1.4.4 DVI as a primary screening test

It is however the potential of using DVI as a primary screening test that has received considerable attention in the past 5 years. There are many potential benefits of DVI as a screening test in low resource settings. DVI can be performed on-site by low-level clinicians or paramedical personnel, is relatively low cost, and because the result is immediately available, a recall system for women with abnormal results is not necessary. Another potential benefit of DVI is that of providing immediate treatment to women with a positive DVI examination.

The equipment required for DVI includes a bed for examination in the lithotomy position, a good light source, a speculum for visualisation of the cervix, a sponge holding forceps, cotton wool for application of acetic acid and a supply of 5% acetic acid. After application of acetic acid, the cervix is inspected for the presence of an aceto-white lesion. No standardised criteria have been developed for defining an aceto-white lesion. Some investigators have attempted to train health workers to grade the intensity and extent of the aceto-white lesion and to introduce low and high thresholds for identifying a significant aceto-white lesion[99]. Others have trained health workers to identify all aceto-white lesions, without attempting to differentiate between low and high-grade lesions[103]. While training health workers in the technique of DVI, training to detect obvious clinical cancers is included.

At the time of designing the study being presented in this thesis, there were few published studies of DVI as a screening test in low resource settings. Megevand et al[103] published data in 1996 on 2426 women who underwent DVI and cytological screening in a peri-urban settlement outside Cape Town,

South Africa. In their study DVI detected 64% of high-grade SIL lesions that were confirmed histologically and missed 11 HSIL lesions in women who had a negative DVI test.

More recently, Sanakarayanana et al[104] published data on 3000 women who were screened by paramedical personnel using DVI and cytology. The screeners were trained to grade the aceto-white lesions: the test was considered positive if any distinct aceto-white area was detected. If the acetowhitening was doubtful or faint, the test was scored as negative. DVI was considered positive in 9.8% of women compared to 10.2% with positive cytology (defined as atypia or worse).

DVI detected 90.1% of the true positive cases (defined as histologically confirmed CIN 2 or worse) compared to cytology which identified 86.2% of the true positives. The approximate specificities of DVI and cytology (just over 90%) were similar as were the positive predictive values (around 17%). In their study, cytology and DVI performed more or less equivalently as screening tests.

The same group[105] reported on a similar study of 1 351 women, aged 22 – 70 years, who were screened, between 1995 and 1997. Of the women screened, 37.7% were considered DVI positive compared to 15.2% of women with positive cytology. Of the 71 women with CIN 2 or worse, 95.8% were detected by a positive DVI test compared to 62.0% that were detected by cytology. Further, DVI detected 25 lesions missed by cytology and cytology detected one lesion missed by DVI. The approximate specificity of DVI was 68% compared to 89.5% for cytology.

The University of Zimbabwe/JHPIEGO Cervical Cancer Project[106] screened 10 934 women in two phases (8731 in phase I and 2203 in phase II) using DVI and cytology. In phase one of the study, 18.1% of women had colposcopy based on a positive DVI or Pap smear. In the second phase of the study, 2147 (97.5%) of the 2203 women underwent colposcopy irrespective of the findings on DVI or cytology. The detection rate of CIN by DVI was similar to cytology and in phase 2 of the study, the sensitivity of DVI for HSIL or greater at 77% was higher than the sensitivity of cytology at 44%. The specificity of DVI for HSIL or greater however, was 64% compared to 91% for cytology.

These studies indicate that DVI may have a role in screening for high-grade disease, although the consistently low specificity reported in a variety of studies may limit its effectiveness as a screening test. The impact of referring large numbers of women for treatment who do not have disease has yet to be evaluated. This thesis will present a detailed analysis of the performance of DVI as a screening test in a low resource setting and will evaluate the outcome of a 'test and treat' policy using DVI as the primary screening test.

### 1.4.5 Cervicography™

Adolf Stafl[107] developed Cervicography™ in 1981. This method of screening involves taking a 35-mm photograph of the cervix (called a Cervigram™), using a specially-adapted camera, known as a Cerviscope™. The cervix is washed with 5% acetic acid and two Cervigrams™ are taken in succession. Cervicography™ was initially developed as an adjunct to cytology, and was not designed to replace cytological evaluation of the cervix. The advantages of the technique are the complementary nature of the test to cytology, and the fact that a permanent visual record of the cervix is obtained.

A number of studies have shown that cytological detection rates of disease are markedly improved by the addition of a second or third test, including Cervicography™[108]. Cervicography™ has been consistently shown to increase the sensitivity of the screening process when used as an adjunct to cytology, largely because it identifies more low-grade lesions than cytology[109].

Schneider et al[110] screened 8460 women with Cervicography™ as a primary screening test and compared the results of Cervicography™ with diagnosis determined by histological analysis and 3 cytological tests. Cervicography™ identified all 11 cancers and cytology missed one. The sensitivity of Cervicography™ for HSIL or cancer was 49.3% overall (specificity 95.0%), 54.6% in women younger than 50 years of age, and 26.9% in women 50 years and older. Cytology had an overall sensitivity for HSIL and cancer of 77.2% (specificity 94.2%), 75.5% in women younger than 50 years of age and 84.6% in women 50 years of age and older. They concluded that Cervicography™ performed marginally better than cytology for the detection of cancer, but worse than cytology for the detection of high-grade precursors.

Nuovo et al[111] conducted an extensive review of all published studies from 1966 - 1996 on the use of cervicography. Twenty-three reports on cervicography were reviewed. After excluding studies in which the reference standard (colposcopy) was not performed on all participants, 7 studies were available for analysis. These are summarised in Table 2. While there is a wide range of test results reported, reflecting different populations of women studied and different referral criteria for colposcopy, all seven studies find that cervicography has a high false-positive rate, with a range of 9.8 – 63.4 % for high-grade lesions reported. In contrast though, cervicography appears to have a high sensitivity for high-grade lesions with sensitivities of over 89% reported in five of these studies. Specificity however was low in all studies. The high negative predictive value (NPV) may indicate some utility of cervicography as a primary screening test. Only one study evaluated cervicography as a primary screening test[117], and found that cervicography had a higher sensitivity than cytology (89 versus 52%, respectively) with similar specificities (92% and 94%, respectively).

Autier et al [119] performed a randomised study of cytology alone versus cytology plus cervicography among 5 550 women aged 18 – 91 years. One year later, women were re-called for repeat screening with cytology and cervicography. All women with positive tests were referred for colposcopy and

histological sampling. The principal endpoint of the study was the rate of histologically confirmed CIN lesions one year after initial screening. At the initial screening round, CIN (of any grade) was histologically confirmed in 0.14% of the group of women who only underwent cytological screening, compared to 0.90% of the women who were screened with both cytology and cervicography.

| Authors | Colposcopic Diagnosis* | Sensitivity | Specificity | False positive rate | PPV | NPV |
|---|---|---|---|---|---|---|
| Index smear diagnosed as 'Atypical' | | | | | | |
| Jones et al[112] | High-grade | 100 | 49.4 | 50.6 | 8.5 | 100.0 |
| | Any grade | 90.4 | 60.4 | 39.6 | 44.3 | 94.7 |
| Schauberger et al[113] | High-grade | 18.2 | 81.6 | 18.4 | 38.6 | 63.8 |
| | Any grade | 19.4 | 82.3 | 17.7 | 38.9 | 63.8 |
| Spitzer et al[114] | High-grade | 100.0 | 36.6 | 63.4 | 10.9 | 100.0 |
| | Any grade | 93.3 | 39.0 | 61.0 | 21.9 | 96.9 |
| Patients referred to Colposcopy clinic with abnormal Pap smears | | | | | | |
| Cecchini et al[115] | High-grade | 95.5 | 48.1 | 51.9 | 6.9 | 99.6 |
| | Any grade | 81.8 | 57.7 | 42.3 | 43.9 | 88.5 |
| Soutter et al[116] | Any grade | 73.0 | 64.0 | 36.0 | 45.5 | 84.8 |
| Screening Population | | | | | | |
| Kesic et al[117] | High-grade | 89.5 | 90.2 | 9.8 | 31.5 | 99.4 |
| | Any grade | 88.9 | 81.8 | 10.6 | 44.4 | 99.1 |
| Patients with condyloma | | | | | | |
| Schauberger et al[118] | High-grade | 100.0 | 53.1 | 48.9 | 13.2 | 100.0 |
| | Any grade | 89.5 | 58.1 | 41.9 | 32.1 | 96.2 |

Table 2: Summary of Cervicography Test Characteristics from Seven Eligible Studies (Reproduced from Nuovo et al [111]) [* Refers to colposcopic grade of dysplasia]

These findings suggested that cervicography substantially increased the detection rate of histologically confirmed CIN in the screening process. However at the second round of screening, the prevalence of CIN in women screened with both cytology and cervicography was reduced (to 0.53%). This may be due to the regression of transient CIN 1 lesions detected initially by cervicography. On the other hand, it is possible that cytology detects different lesions from those detected by cervicography, and that cervicography- detected lesions do not express the cellular abnormalities necessary for detection by cytology and vice versa. Hence, cervicography and cytology are complementary to one another.

In the study presented in this thesis, Cervicography™ was evaluated as a primary screening test. In addition, Cervicography™ it was used as a form of quality control for DVI as it provided us with a photographic representation of each woman's cervix to ensure that no cancers were missed.

### 1.4.6 HPV DNA testing

The role of HPV DNA in the aetiology of cervical cancer has been discussed, as has the role of the persistence of oncogenic type of HPV DNA infection of the cervix in developing progressive SIL and cancer. The powerful association of certain types of HPV infection of the cervix with SIL and cancer has prompted an investigation into the role of HPV DNA detection in primary and adjunctive cervical screening.

Two methods are used for the detection of HPV DNA: those that identify nucleic acids directly and those that amplify nucleic acids first and then detect the amplified product. The latter method, know as polymerase chain reaction or PCR-based techniques is considered the 'gold-standard' for HPV DNA detection. Direct identification of nucleic acids can be performed using Southern Blot, filter-in-situ hybridisation (FISH), *in-situ* hybridisation, Dot Blot and a more recent method, known as Hybrid Capture™.

For HPV DNA testing to be used in a mass screening programme, Cuzick et al[120] have suggested that the method used for HPV testing should fulfil the following criteria:

1. The test must be readily available
2. Highly sensitive and specific for a broad spectrum of high-risk types of genital HPV
3. Capable of using minimally invasive sample types (e.g. cervical brushes as opposed to tissue samples)
4. Possess high level of intra-and inter-laboratory reproducibility
5. Suitable for high-volume testing (e.g. 96-well microtitre plate)
6. Potential for full or semi-automated execution of the tests
7. Cost-effective execution within a large-volume screening programme

Southern blotting has been widely used for the identification and typing of HPV and in expert hands, is sensitive, specific and reproducible. However, the test requires relatively large amounts of DNA such as would be obtained by tissue samples e.g. from a cervical biopsy. It is also labour intensive, time-consuming and not amenable to automation and is therefore not a suitable technique for screening. Dot Blot is simpler and quicker to execute than Southern Blotting. Two commercially available Dot Blot systems that have been widely reported in the literature are the Virapap and Viratype® kits (Life Technologies). In general, these techniques have lower sensitivity and specificity than Southern blotting and the requirement for large amounts of input DNA, make these techniques unsuitable for screening. FISH is simple to execute but is neither sensitive nor specific and has been largely abandoned for clinical applications.

New *in-situ* hybridisation protocols are currently being developed that can be used to detect and type HPV DNA in standard cervical smears and automated systems are being designed which could allow the processing of large numbers of samples. This technique is still under development and not yet

commercially available, however, it may be an attractive option for HPV DNA detection in screening protocols in the future. Similarly, while the PCR-based techniques are considered the 'gold standard' for HPV DNA detection and typing, there are no commercially produced kits, although these are currently under development. In addition, the PCR process needs to be conducted in a facility designed to prevent the contamination of samples with previously amplified products. While this requirement can be easily overcome in specialised laboratories, this would pose considerable logistic problems in the context of using PCR techniques in a large-scale screening programme.

The original Hybrid Capture™ (HC) technique, known as HC I, was chosen as the technique for HPV DNA detection in this study, as at the time, the test was commercially available and was considered the most appropriate test in the context of a large-scale screening project. The test is easy to perform and initial training of technologists is relatively simple. In addition, the laboratory requirements are considerably simpler than those of PCR-based techniques, as the test does not rely upon amplification of the target material to achieve its sensitivity.

During the course of the study, a new generation of the Hybrid Capture™ test was introduced, known as HC II, which has an additional 4 probes for the detection of HPV types and uses a microtitre format enabling large numbers of samples (up to 96) to be processed at one time. Both tests are suitable for large-scale screening programmes, although the analytical sensitivity of HC I is reported to be less than that of HC II[121]. HPV DNA detection by HC II has been shown in one study to have near equivalent ability to the PCR system based on the MY09/11 primer[122].

Hybrid Capture I, is a quantitative test, which is available in standardised kit format. The test is an in-vitro, solution hybridisation, signal amplification test for detecting DNA or RNA targets. In this assay, cellular DNA is extracted, denatured in an alkaline solution and then hybridised with complementary RNA probes to produce DNA-RNA molecules. The hybrid DNA-RNA molecules are then removed from solution by antibodies that coat the walls of the tube. These antibodies only recognise the three-dimensional structure of the hybrid DNA-RNA molecules and double-stranded DNA or single-stranded RNA molecules are removed. The presence of the hybrid molecules is detected by the addition of anti-hybrid antibodies labelled with alkaline phosphatase, which binds to the immobilised target hybrid molecules. The alkaline phosphatase is then reacted with a dioxetane chemi-luminescent substrate to produce light, which is measured by a luminometer. Results are then expressed as relative light units (RLUs), which are a measure of the light produced by the individual sample reaction divided by the mean level of light generated by three 1.0pg/ml positive calibrators. As such, a reading of 1.0 RLU is equivalent to 1.0pg/ml.

While having a generally lower analytical sensitivity than HC II, HC I has been shown in some studies to have a similar sensitivity to PCR for the detection of HPV DNA. Sun et al[123] assayed cervical samples for HPV DNA from 520 women using HC I and PCR and they found a good correlation between HC I and PCR. HC I correctly identified 92% of samples found by PCR to contain a high-risk

type of HPV. Cope et al[124] compared PCR using the MY09/11 primer system with HC I for the detection of HPV in 499 cervicovaginal lavage specimens from women with normal cytology and 97 specimens from women with varying degrees of SIL. While the two tests detect a different range of HPV types, when the tests were compared on the basis of the 14 HPV types detected by both methods, PCR detected 108 positive samples compared to 79 detected by HC I. Overall however, the two methods agreed 93% of the time on whether a specimen was positive or negative for one of the 14 types detected by both methods, and 97.2% of the time, they agreed on whether the specimens were positive for cancer-associated types of HPV.

Schiffman et al[125] showed that inter-laboratory agreement in three laboratories that used HC I was good. Inter-laboratory agreement rates on HPV positivity in a study of 199 cervical specimens for either high risk or low risk types ranged from 87 – 94% and kappa values ranged from 0.61 to 0.83.

The advantages of HPV DNA testing include the following: HPV DNA detection is an objective test, the laboratory procedures use standardised kits, are relatively simple and can be performed by mid- to low-level technicians. In addition, large numbers of tests can be performed per day compared to the limited number of cervical smears that can be read per day by highly trained cytotechnologists. Finally, with the rapid development of modern molecular technology, it may become feasible to develop a fully automated method of HPV DNA detection using the hybrid capture format. The ultimate however, would be to develop a method of HPV DNA which could be used in the clinic as a side-room test, such as the dipstix tests developed for pregnancy testing.

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# Chapter Two

# Methods

## 2.1. Introduction

### 2.1.1 Overall Aim of the Study

The study was designed to evaluate alternatives to cytology for screening for cervical cancer and its precursors. The target population was women aged 35 – 65 years, who had not been screened previously and who lived in a low-resource setting.

### 2.1.2 Objectives of the Study

1. To compare the sensitivity, specificity, positive predictive value and negative predictive value of the following three cervical screening tests with those of cervical cytology:

- Direct visual inspection (DVI) of the cervix with the naked eye and with low magnification after the application of 5% acetic acid.
- Detection of high-risk or oncogenic types of Human Papillomavirus (HPV)
- Cervicography™ (35 mm photographs of the cervix taken after the application of 5% acetic acid)

2. To analyse information on the performance of the four screening tests and the prevalence of disease in the study population to predict expected outcomes of each test used alone or in various combinations of tests.

3. To determine, in a low resource setting, the success rate, short and intermediate term complication rates of LEEP as a treatment for preinvasive disease of the cervix and to evaluate LEEP when used in a 'see and treat' mode i.e. treatment of preinvasive lesions of the cervix with LEEP after colposcopic assessment but without prior histological evaluation

4. To describe the process of setting up a cervical cancer screening study in the context of a community-based research project.

### 2.1.3 Why Khayelitsha was chosen as the study site – a Political and Historical Perspective

Khayelitsha was chosen as the study site as it is a low-resource area, only 20 kilometres outside of Cape Town, where most people live in poverty, with poor levels of health in general and relatively poor health care services. Consequently, we expected to find a high level of preinvasive disease and a large group of unscreened women. Placing the study in this area enabled us to evaluate the different screening tests within the context of low-resource community. In addition, the project was able to provide screening services to women who would not otherwise have had access to screening. This section provides the reader with a history of the development of Khayelitsha and describes the socio-demographic characteristics of the community, in order to place in perspective the conditions in which the study was performed.

Khayelitsha is a peri-urban settlement that was established in 1983, and is inhabited almost entirely by African people. The movement of African people in South Africa was controlled and restricted since the early 1900s. This policy was intensified after 1948 when the now infamous policy of Apartheid became government policy. The essence of the policy of Apartheid was to develop South Africa along racial lines with the intention of creating separate states for white and black South Africans. This policy resulted in the creation of 9 areas in South Africa that were designated as 'homelands' and which were supposed to become independent countries, populated exclusively by black South Africans. These 9 areas, created along tribal lines, represented approximately 13% of the land of South Africa and were designed to accommodate over 75% of the population[1].

The implementation of this policy began in earnest in the early 1960s and resulted in over three and a half million African people being forcibly displaced and moved to the 'correct' areas by the early 1980s[2]. The intention was to move African people out of 'white rural' and 'white urban' areas and to force them to move to the 'homelands'. The extent to which migration to the cities by African people was allowed, was determined by the need for their labour. This process was governed by a complex set of laws that included the 'Pass Laws'. The 'Pass Laws' decreed that Africans had to carry a 'reference book' at all times to indicate whether they were 'legally' or 'illegally' resident or working in a particular area.

Despite the rigorous application of these laws many African people did migrate to the cities[2]. These laws coupled with the deliberate failure to provide housing for African people in the cities forced large numbers of African people to live in squatter camps, in and around South African cities.

In Cape Town (capital of the Western Cape), in the early 1980s, there were 3 established 'legal' townships in which African people were allowed to live and work. In addition, large numbers of African people had managed to migrate to the city and were living in squatter camps. It was government policy at the time that the Western Cape should be retained as the 'traditional place of

residence and employment of the White and Coloured* communities'. Further, it was stated that the 'uncontrolled influx of Black people to the Cape Metropolitan Area should be countered as far as possible'[3].

In 1983, the proposed development of Khayelitsha was announced. The intention was to move 'legal' Africans living in established townships in Cape Town or in squatter camps to Khayelitsha. 'Illegal' Africans were to be forced to move to the 'homelands' of Transkei and Ciskei (Transkei obtained 'independence' in 1976 and Ciskei in 1981) [4,5]. In 1984 however, there was widespread political violence in many of the squatter camps in Cape Town. This resulted in thousands of people being displaced from their homes and many people were forced to settle in Khayelitsha, in far larger numbers than were intended by the State. By the end of 1984, 30 000 people had moved to an area of Khayelitsha that became known as Site C and established the first informal settlement. By September 1986 the total population of Khayelitsha had reached approximately 120 000 people.

In 1986 the 'Pass laws' were repealed. This, together with the widespread poverty that had been created by the 'homeland' system, stimulated a large migration of rural people to urban areas. By 1996, the total population of Khayelitsha had grown to between 350 000 – 500 000 people[4].

### 2.1.4 Socio-economic and Demographic Profile of Women living in Khayelitsha

Khayelitsha is divided into various towns, where people live in brick houses and 'sites', where people live in 'site and service' schemes. The 'site and service' schemes provide a plot of land with an outside tap for running water, a flush toilet, and refuse removal. The 'houses' or 'shacks', as they are known, are erected by the people themselves using materials such as wood, plastic and corrugated iron. Since 1994 when a new government was elected and the policy of Apartheid was formally terminated, many of the shacks have acquired electricity. A smaller but rapidly increasing part of Khayelitsha is known as the 'unserviced sites' where people have moved onto vacant land and erected 'shacks' without any infrastructure, running water or sanitation.

The socio-economic and demographic profile of the population of Khayelitsha was described in 1991 by Cooper et al[6]. The study reports on interviews of respondents living in 722 households in Khayelitsha in 1989; 91.3% of the respondents were women. Of the female respondents interviewed, 13.7% lived in formal housing, 54.3% lived in serviced sites and 31.7% lived in unserviced sites. Seven per cent of the respondents had no formal schooling, 39% had primary school education and 54% has some secondary school education.

The majority of the respondents (71%) had been born in a 'homeland' and 55.3% still regarded the 'homeland' as home. In addition, 67.3% of women had a dwelling in the 'homeland', 41.2% had

* Coloured people refers to people of mixed descent

children living in the homeland and 86.9% had relatives living in the 'homeland'. Over 80% of the respondents had moved to Khayelitsha from a rural area and the primary reasons for migration were to work and to join a spouse.

Of the female respondents, 45% had no employment in either the formal or the informal sector; 67% were unemployed if employment in the informal sector was excluded. Of the women who were employed, 66.2% were employed as domestic workers. Among both formal and informal sector employees, 80% earned less than R100 per week, while 89% of formal sector employees earned under R400 per month.

The social and environmental conditions in Khayelitsha reflect the inequities created by the Apartheid system in South Africa and the problems of rapid urbanisation. Poverty, overcrowding, poor quality housing, inadequate sanitation and water, high levels of crime and violence and pervasive ill health characterise the area and its population[7].

For example, the incidence of Tuberculosis in Khayelitsha was estimated to be 543 per 100 000 population in 1996 (compared to the national prevalence of 223 per 100 000 population)[7]. The prevalence of HIV infection in women was about 10% (derived from anonymous screening of women attending antenatal clinics in Khayelitsha). The infant mortality rate (IMR) for the area was estimated as 38.3 per 1000 live births in 1996. In 1994, the national IMR was 48.9/1000 and 54.3/1000 for the African population. Due to poor record keeping in Khayelitsha the IMR is probably an underestimate of the true rate.

Figure 5 shows photographs of the typical housing found in Khayelitsha.

### 2.1.5 Study site: Nolungile Clinic

Site C was chosen as the study site because of its central location in Khayelitsha and the close proximity of Site C to taxi ranks, bus stops and a railway station. In 1996, Site C had a population of approximately 70 000 people living in about 7600 serviced shacks and 640 unserviced shacks[7]. The study site was Nolungile Clinic, which had been established in 1983. Nolungile clinic is situated in the centre of Site C and is within walking distance of all transport facilities and most people's homes.

## 2.2 Preparations for the Study

### 2.2.1 Development of Community Participation

Four months prior to establishing the project, all Community Health Forums (CHFs), primary health care (PHC) services and non-governmental organisations (NGOs) working in the Khayelitsha area were contacted and the nature of the proposed study was explained to members of each organisation.

The CHFs were established after the 1994 elections in South Africa and consisted of elected representatives from the community who were mandated to monitor the provision of health services in their area. Obtaining permission to begin projects in the Khayelitsha area from these community health forums was an essential step in ensuring community approval and participation.

Each section of Khayelitsha has it's own CHF, and five of these were contacted, including the CHF of Site C. These forums first required a meeting with the project investigator during which the nature of the project was explained in detail. Executive members then presented this information to the wider community at mass meetings. At these meetings members of the community had the opportunity to ask questions and to discuss the nature of the project. I attended one of these mass meetings and explained the rationale behind the project and it's advantages to the women who would be screened. There was an enthusiastic approval for the project.

Meetings with the Association for Traditional Healers in Khayelitsha were also arranged. Many people in Khayelitsha use traditional healers as their primary or supplementary health care resource, hence the importance of ensuring their approval and co-operation with the project. Two separate meetings were held with local traditional healers. The group gave their approval and agreed to co-operate with the project by referring their patients for screening where appropriate. The CHFs and Traditional Healers required feedback of the results of the research project and I reported to the respective executive committees every six months.

### 2.2.2 Staff and Training

### 2.2.3 Staff

The staff consisted of two female community health workers (CHW) who had previously worked on a cervical screening project, who knew the area well and were Xhosa speaking (the predominant language of the women resident in Khayelitsha). In addition, a senior nursing sister was employed. She had previously been trained in direct visual inspection (DVI) of the cervix, had worked in the area and was Xhosa-speaking.

A full time medical officer was employed to oversee the non-research related medical problems of the women recruited to the study, to manage the data and to be trained in colposcopy and the management of preinvasive lesions of the cervix.

### 2.2.4 Training

Training of staff began in December 1995 and was completed midway through January 1996. The nursing sister was re-trained in DVI and in the exact protocol for examination of the women over a period of one week immediately prior to initiating screening. Her training consisted of lectures on the natural history of preinvasive lesions of the cervix and of cervical cancer, and included an extensive review of slides of normal and abnormal cervices, before and after the application of acetic acid. She was trained to identify all aceto-white lesions without attempting to distinguish minor from more significant lesions.

Fred Kosteki from National Testing Laboratories, USA visited Cape Town and trained the nursing sister, medical officer and author in all aspects of Cervicography™. The author, a senior specialist in the Gynecology Oncology Unit, UCT and two technologists from Department of Pathology, UCT were trained in the technique of HPV DNA testing using Hybrid Capture I™ by Thomas Wright, Associate Professor, Division Gynecological Pathology, the Department of Pathology, Columbia University, New York.

### 2.2.5 Study Facilities

At Nolungile Clinic, a room was provided for the examination of the women and the Cancer Association of South Africa provided the project with a fully equipped mobile caravan, which was parked in an empty plot next to the clinic. The caravan was not used as a mobile screening clinic, but since it was equipped with a colposcope, a generator and the equipment to treat women with preinvasive lesions of the cervix, it was decided to use the caravan as the diagnosis and treatment 'room'. This provided extra space and greater privacy. The generator was used as a back up to the frequent electricity failures experienced by the clinic.

The facilities at Groote Schuur Hospital (GSH), a large tertiary hospital in Cape Town, were made available for the referral of women who participated in the study and were incidentally found to have tertiary gynaecological or other medical problems. Women with cervical cancer were referred to GSH for further management. The facilities at Nolungile Clinic were utilised for women with primary health care problems.

## 2.3 Inclusion Criteria

The target population for this screening study was a volunteer sample of women living in Khayelitsha who had not been previously screened and were aged 35 – 65 years. This particular age group was selected in order to screen the most high-risk women in the community. A number of large cohort studies indicate that the prevalence of high-grade preinvasive lesions (CIN 2 or worse) is highest in women over the age of 35 years. Miller et al[8] reported on 3 cohorts of women screened in the British Columbia study which included over 300 000 women screened. Their data confirmed that the incidence of high-grade preinvasive lesions of the cervix peaks at around age 35 years, but continues to occur to an appreciable extent in women in their 60s. The study by Baillie[9] in the Western Cape confirms these data with the highest prevalence of CIN 2 and 3 found in the over 35 age group.

Inclusion criteria were:

- Women aged 35 – 65 years
- No history of having been screened previously

Exclusion criteria:

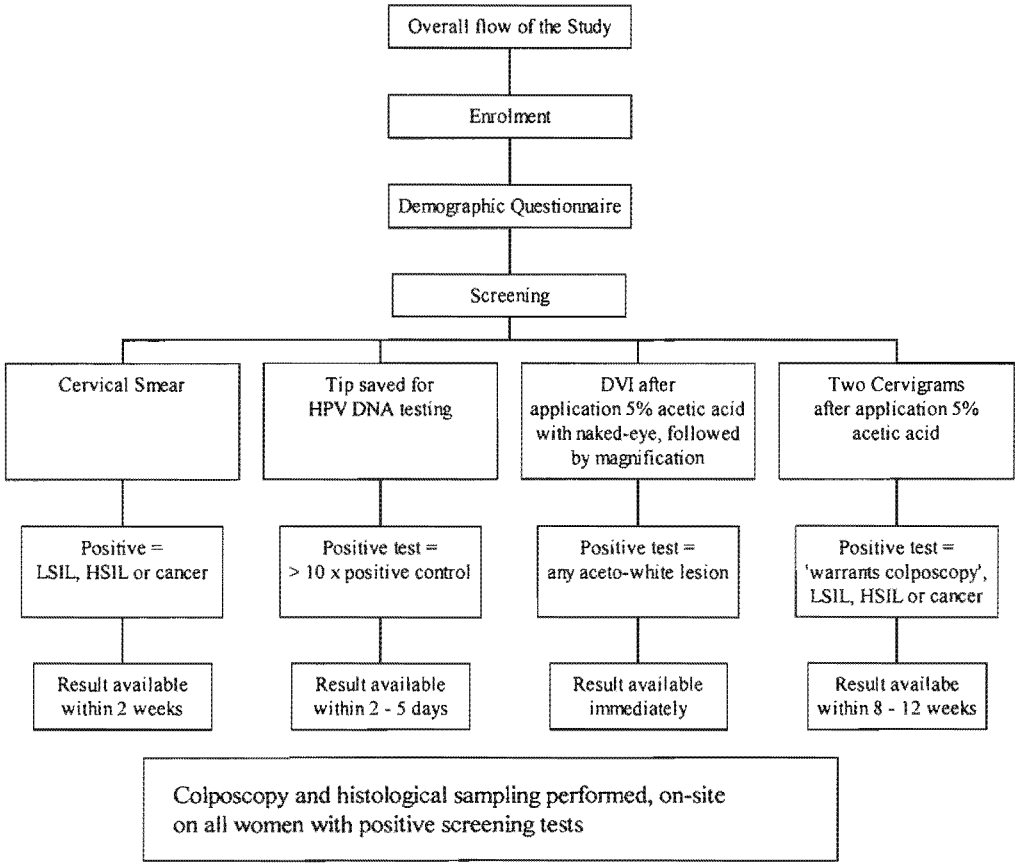
- Previous hysterectomy or previous treatment for cervical disease
- Pregnant women
- Women unable to give informed consent

## 2.4 Overall Design of the Study

### 2.4.1 Algorithm of the Study Flow

An overview of the study is illustrated in Figure 6. This was a cross-sectional, community based research study. Women were recruited from the wider Khayelitsha area between January 1996 and September 1997.

Figure 6: Algorithm of Study Flow



Women were recruited to the study from Site C and the wider Khayelitsha area, by one of the CHWs. Health care clinics, shopping centres, bus stops and railway stations throughout Khayelitsha were visited in rotation on a daily basis. In addition, central points in residential areas were visited and women were informally invited to listen to the CHW explain about cervical cancer prevention. These 'informal' meetings in the streets were well attended by women and men

Extensive use was made of local and national radio and local media to inform women about the project and the advantages of cervical cancer prevention. For instance, the local Khayelitsha radio station, Zibonele Radio, interviewed various members of the project (the author, the nursing sister and one of the CHWs) on a regular basis throughout the 19 months of the project. These interviews, which focused on explaining the nature and rationale of cervical cancer prevention, were followed by 'phone-in' programmes where women were invited to ask questions.

The 'phone-in' programmes provided the project team with insight into women's fears and misconceptions around cervical cancer and its prevention. It became clear that the concept of 'prevention' was poorly understood and enabled us to improve the language we used and our methods of explaining cervical cancer prevention. In addition, it gave the project team the opportunity to provide women with information on health issues not directly related to cervical cancer prevention, such as AIDS, vaginal infections and sexually transmitted diseases, domestic violence and rape, tuberculosis and the signs and symptoms of other cancers, such as breast cancer.

The author also appeared on national radio, specifically on a woman's magazine programme, which is run by a widely popular radio personality. The programme had an estimated audience of one million women nationally and took the same format as the local Zibonele radio programme. After listening to the many questions and concerns women expressed during the 'phone-in' parts of the programme, the presenter, the medical officer of the project and the author organised a 'health day' in Khayelitsha in November of 1996. We invited health workers and services from different areas of Khayelitsha to collaborate on the project. Health information on cervical cancer prevention, AIDS, maternal and child health, tuberculosis and prevention of sexually transmitted diseases was presented using theatre, poetry, songs and informal lectures. A competition was arranged which required women to answer the question, 'How is cervical cancer prevented?' and over 1000 women from Khayelitsha entered the competition. The whole day was videotaped and recorded and was subsequently replayed on the national radio programme. The video was edited and has been shown to women attending for screening or colposcopy on a weekly basis.

The author also wrote short articles on women's health and cervical cancer prevention for the local Khayelitsha newspaper. These articles were printed in English and Xhosa and appeared at approximately 3 monthly intervals. The author organised and participated in numerous seminars and workshops directed at primary health care physicians and nurse practitioners working in Khayelitsha.

These workshops were aimed at increasing awareness about cervical cancer prevention and the management of gynaecological problems at the primary health care level.

To ensure that the women attending the project understood the information being provided by the project personnel, a series of focus groups were organised with women who had already been screened in November 1996 through to March 1997. These focus groups, conducted by a trained Xhosa speaking interviewer, explored women's understanding, misconceptions and fears. From these meetings, the educational material and the method of explaining cervical cancer prevention was refined and developed.

Although the impact of the educational material was not formally evaluated in the study, it was our observation that participation in the project increased as the nature of our educational material improved. It appeared that a clear understanding of the purpose of screening reduced anxiety and provided a strong incentive to women to participate in the study and to return for results and follow up. The focus groups also gave us insight into the role of the extended family in Xhosa culture. Women rarely take important decisions alone. Rather it is custom for women to consult with male and older female members of the family before accepting treatment, particularly if the treatment requires surgical intervention. With this information, we included men and older women in our educational initiatives and encouraged women to bring their significant family members to our educational talks.

Further, we learnt that there was great distrust of the use of surgical methods for the treatment of cancer, as it is believed that cancer is a 'poison' that needs to be drawn out of the body[10]. 'Cutting' through cancer is believed to stimulate the tumour to grow. This alerted us to the need for very careful explanation about treatment of preinvasive lesions to dispel fears that we were treating, and possibly aggravating, cancer.

It was also learned that most women recruited to the study consulted with traditional healers and were under the impression that the project team would disapprove of this. We therefore made a specific point of explaining that we did not disapprove of the use of traditional healers and encouraged women to integrate our methods of diagnosis and treatment with those used by traditional healers.

### 2.4.3 Informed Consent and Enrolment

At enrolment, the nature and purpose of the study was outlined on a one-on-one basis to the women, by the CHW responsible for enrolment. Eligibility criteria were checked. Informed, written consent was taken. The consent form was translated into Xhosa by a senior lecturer in Xhosa language at the University of Cape Town.

Thereafter the women were assigned a unique study number and their names and numbers entered into an enrolment log. The clinical examination form, the cytology request form, the cytology slide and the

HPV DNA specimen collection tube were labelled with the name and patient number of each woman by the enrolment CHW.

In addition, their contact details were documented and a detailed, structured questionnaire (appendix A) was administered. The questionnaire elicited information on socio-economic and demographic factors, sexual, reproductive and contraceptive history, and life-style habits such as tobacco and alcohol use. The questionnaire was administered by the enrolment CHW and took approximately 10 minutes to complete. The medical officer checked the questionnaire on a daily basis to ensure completeness of the data.

Each woman was given a patient card which recorded her name, patient number, date of birth, address, date of enrolment and date for return for follow up. At the back of the card, space was provided for clinical findings by the screening nursing sister, the results of the screening tests and the colposcopic examination.

### 2.4.4 Screening

The nursing sister checked that the forms, slide and specimen collection tube were correctly labelled with the woman's name and unique study number. The nature of the screening process was explained in detail. Women were examined in lithotomy.

The gynaecological examination began with the visual examination of the vulva. Thereafter a bi-valve speculum was passed and the vagina inspected for discharge, which was graded as mild, moderate or severe. This was followed by naked eye inspection of the cervix and documented the presence of inflammation, atrophy, ulcers, polyps and obvious cancer. The cervix was illuminated by using an electrical headlamp.

A cervical smear was obtained using an Accellon Combi cervical biosampler (MedScand, Hollywood, FL) for the first 1200 women screened. Once the slide had been prepared for the cervical smear, the tip of the Accellon sampler was broken into an HPV DNA specimen collection tube (Digene Corporation, Silver Spring, MD).

After the first 1200 women had been screened, the cervical sampling device was changed to an Aylesbury spatula and an endocervical brush. The Aylesbury spatula was first used to obtain an ectocervical smear and immediately placed on the glass slide. The endocervical brush was then inserted into the endocervical canal, turned once and immediately placed over the same glass slide and sprayed with fixative. The tip of the endocervical brush was then placed in the HPV DNA specimen collection tube.

After the cervical smear had been performed, the cervix was washed with 5% acetic acid using cotton-wool balls soaked in acetic acid. While waiting for the acetic acid reaction the details of the vulval, vaginal and cervical examination were recorded on the clinical examination form. The cervix was inspected with the naked eye for the presence of an aceto-white lesion. Acetic acid was then applied for the second time and the cervix re-examined for the presence of an aceto-white lesion using a 2.5x hand-held monocular lens, also known as a Gynescope (Selsi, Edmund Scientific, Barrington, NJ, USA).

Acetic acid was applied for the third time and two Cervigrams™ (i.e. 35-mm photograph of the cervix taken with a specially adapted camera called a Cerviscope™) were taken.

At the end of the examination, the clinical examination form and the cervigram log were completed. The medical officer checked these forms on a daily basis to ensure completeness of the data.

### 2.4.5 Referral for Colposcopy

Women were referred for colposcopy if any one of the screening tests (DVI, with or without magnification, positive Cytology, positive HPV DNA test or Cervicography™) were positive. Women who had lesions noted on naked-eye examination of the cervix (prior to the application of 5% acetic acid) were referred for colposcopy at the discretion of the nursing sister, as naked-eye inspection of the cervix without the use of acetic acid, was not considered a screening test.

Women were informed of the result of the clinical examination and were asked to return for outstanding results and/or colposcopy within 2 – 6 days of being screened.

At the end of each day, the forms, slides, specimen collection tubes, film and Cervigram™ logs were placed in separate boxes, counted and entered into a checklist by one of the CHWs. These were delivered on a daily basis to the project office, located at the CANSA, and were counted and checked by the medical officer to ensure that the labelling and number of specimens and forms tallied correctly.

No formal method of quality control of the gynaecological examination by the sister was included in the study. However, informal quality control consisted of regular retraining sessions (on a 4 monthly basis) of the nursing sister, and by assessing the appropriateness of referrals at the colposcopic examination.

### 2.4.6 HPV DNA Testing

HPV DNA status was determined at the University of Cape Town using the first generation Hybrid Capture HPV DNA Assay (Digene Corporation, Silver Spring, MD). The test was run according to the

manufacturer's protocol using the tube-based format and probes for 'high oncogenic risk' HPV tyes (i.e., types 16, 18, 31, 33, 35, 45, 51, 52 and 56).

HPV determinations were quantitative, and women with samples producing readings greater than or equal to 10 times (x) the positive control (10 pg/ml or 100 000 HPV genome copies per test) were referred for colposcopy. Women who had a positive HPV DNA test but less than 10 x the positive control were not referred for colposcopy on this basis. However, women with a positive HPV DNA test but less than 10 x the positive control and who were positive on one of the other screening tests did undergo colposcopy. The data on these women is included in the data analysis for the purpose of comparison.

The HPV DNA test was run on a weekly basis and women were given their results within 2 – 6 days of the test being performed. The results of the HPV DNA test were entered into a coded data sheet.

One year after the study began, Digene Corporation (Silver Spring, MD) introduced the new generation assay for the detection of HPV DNA, known as Hybrid Capture II (HC II). A sample of Accellon cervical samplers that had been stored at 4°C were re-tested later at Columbia University using the second generation HC II assay. The sample consisted of all women with a histological diagnosis of low-grade SIL, high-grade SIL, or invasive cervical cancer and an approximately 10 percent random sample (n=243) of women with no positive screening test results or with a positive screening test(s), but no SIL or cancer detected at colposcopy and biopsy. HC II uses probes for 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and a microtitre plate-based assay format. The chosen analytic sensitivity limit of HC II for high-risk HPV types was 1 pg/ml.

### 2.4.7 Cervical Cytology

The University of Cape Town (UCT) Cytopathology laboratory evaluated cervical smears according to the routine protocol of the laboratory. Cervical smears were classified using the Bethesda System terminology. The different diagnostic categories used are shown in Table 3.

All slides were initially evaluated by cytotechnologists. The cytotechnologists rapidly re-screened 100% of each other's slides. Two senior cytotechnologists then individually re-screened a random sample of 10% of the normal smears as well as all smears taken from postmenopausal women. All slides identified as abnormal (ASCUS, AGUS, LSIL, HSIL or malignant) were re-screened by the same two senior cytotechnologists. The senior cytotechnologists referred cervical smears with a diagnosis of AGUS, LSIL, HSIL or malignancy to a Cytopathologist for review. ASCUS smears were referred to the Cyopathologists at the discretion of the senior cytotechnologists.

| Diagnostic Category | Abbreviation and explanation |
|---|---|
| Satisfactory for cytological assessment | |
| Satisfactory but limited by | SBLB (e.g.: lack of endocervical component, obscuring blood or debris, scanty cellular material) |
| Unsatisfactory | Inadequate for cytological evaluation |
| Within normal limits | WNL |
| Benign Epithelial Changes | Infection, inflammation, atrophy, reparative changes |
| Atypical Squamous Cells of Unknown Significance | ASCUS |
| Atypical Glandular Cells of Unknown Significance | AGUS |
| Low grade Squamous Intraepithelial Lesions | LSIL = includes lesions previously called CIN1 or cytological changes of HPV infection |
| High grade Squamous Intraepithelial Lesions | HSIL = previously called CIN 2 or CIN 3 |
| Malignant or suspicious of malignancy | |

Table 3: Classification of Cervical Cytology using Bethesda system of 1992

Cervical cytology results were entered into a coded data sheet. Women with a cytological diagnosis of LSIL, HSIL, suspicious of malignancy or malignant were referred for colposcopy within two weeks of being screened.

### 2.4.8 Cervicography™

The 35-mm rolls of film were sent to National Testing Laboratories (NTL), USA for processing and evaluation. The film was developed by NTL according to their specifications and each Cervigram™ Slide was reviewed for quality after processing. The Cervigram™ Slides were sent to a NTL evaluator. Cervigram™ Slides were projected onto a screen and were approximately six feet in width. Once projected onto the screen, the Cervigram™ Slides were evaluated from a distance of 1 - 3 feet. After the initial evaluation, 100% of the Cervigram™ Slides were re-evaluated by a second evaluator, blinded to the results of the first evaluation. In the case of a discrepancy, the slide was reviewed in conference and the evaluation of the conference was accepted as the final diagnosis.

The classification of the Cervigram™ results is illustrated in Table 4 and Figures 7 - 10 show photographs of Cervigram™ Slides illustrating 4 different diagnostic categories. 'Atypical' refers to the presence of an aceto-white change on the cervix but based on its site and morphology, colposcopy is not considered necessary. 'Warrants colposcopy' indicates that no definitive lesion is seen but the appearance of the cervix warrants colposcopic assessment to exclude significant disease. 'Technically

defective' means that the Cervigram™ Slide is not adequate for evaluation either due to the quality of the slide or the cervix is obscured by the speculum, blood or mucous.

'LSIL' refers to an aceto-white lesion with a predicted histological diagnosis of LSIL (HPV or CIN 1). 'HSIL' predicts a histological diagnosis of HSIL (CIN 2 or CIN 3) and 'malignant' predicts cancer.

Women were recalled for colposcopy if the cervigram result was 'warrants colposcopy', LSIL, HSIL or malignant. The results of the Cervigrams™ were available within 8 to 12 weeks of the test being performed.

| Diagnosis | Cervigram™ Terminology |
|---|---|
| Normal<br>TZ visible<br>TZ not visible | <br>N1<br>N2 |
| Atypical (lesion inside or outside TZ) | A1 or 2 |
| Warrants Colposcopy | P0 |
| LSIL (with or without TZ visible) | P1A or B |
| HSIL | P2 |
| Cancer | P3 |
| Technically Defective or unevaluable | TD |

Table 4: Diagnostic Categories for Evaluation of Cervigram™ Slides
[TZ = transformation zone]

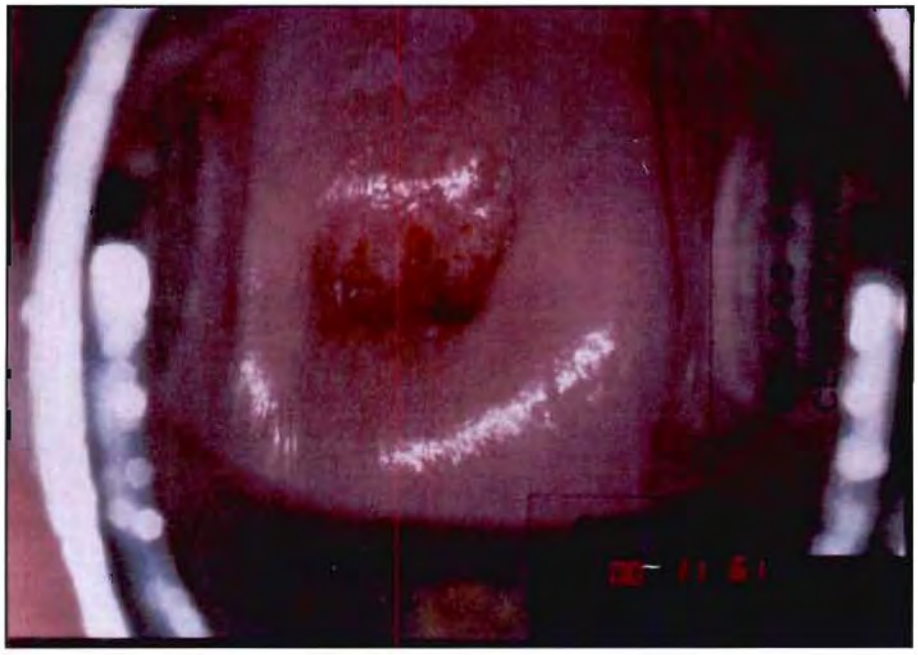


Figure 7: Cervigram evaluation - Squamous Metaplasia

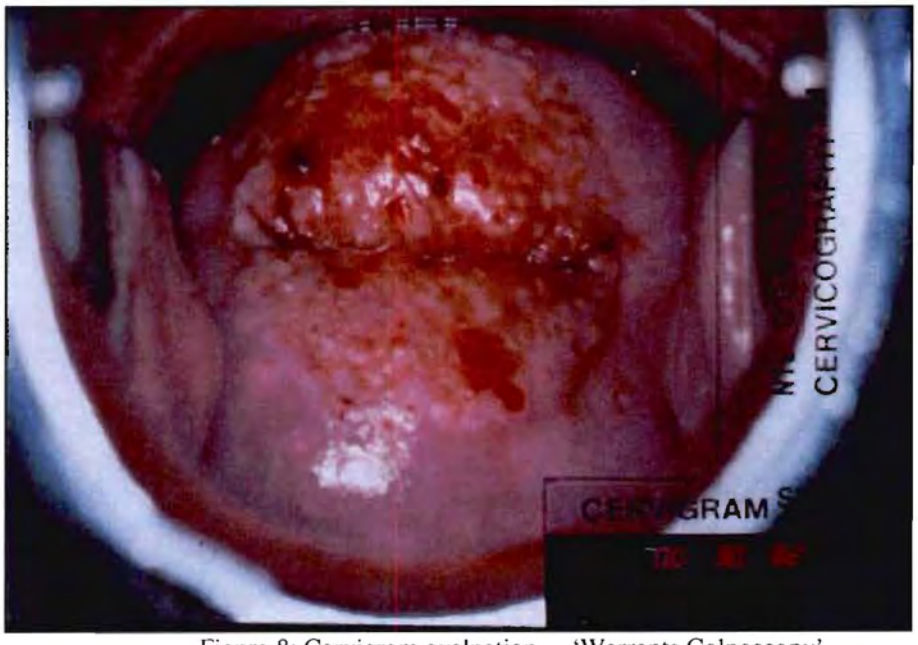



Figure 8: Cervigram evaluation – 'Warrants Colposcopy'

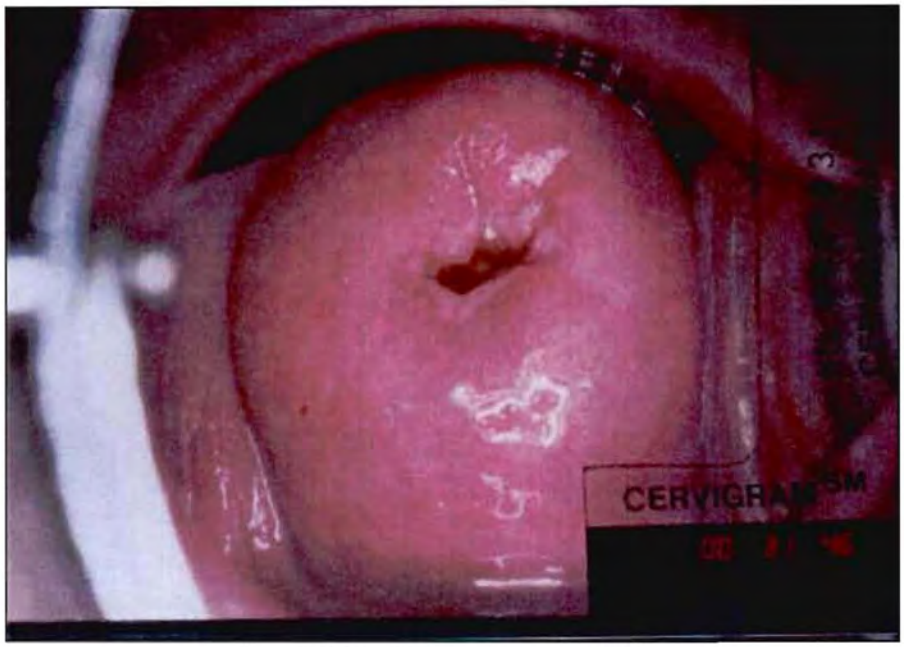


Figure 9: Cervigram evaluation - LSIL

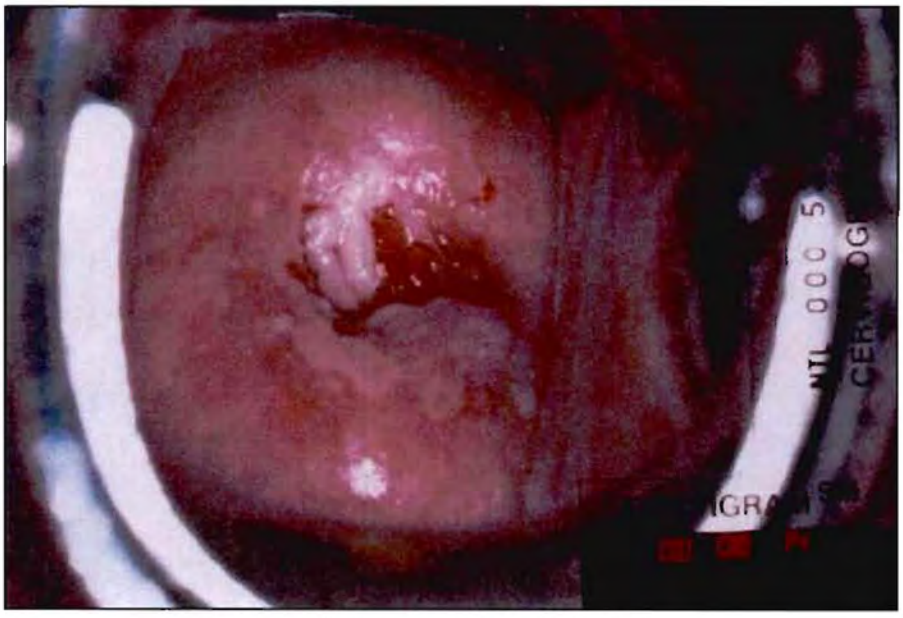


Figure 10: Cervigram evaluation - HSIL

## 2.5 Diagnosis and treatment

### 2.5.1 Referral for colposcopy

Women were referred for colposcopy if any of the four screening tests were positive. Women who were DVI positive or HPV DNA positive to 10 x the positive control were referred for colposcopy within 2 – 6 days of being screened. All colposcopies were performed blinded to the result of the cervical smear, unless a positive Pap was the only reason for colposcopic referral.

Women were informed about the delay in the cytology result and were given a date to return for results two weeks after being screened. Due to the longer delay in the Cervigram™ result (8 – 12 weeks), women were informed that they would be contacted by one of the CHWs if the Cervigram™ result was positive and they had not already undergone a colposcopic examination on the basis of other positive screening tests. To ensure that women were contactable, each woman's address or the address of a relative or friend was double-checked at each visit.

### 2.5.2 Colposcopic Examination: Reid's Colposcopic Index

The author performed or directly supervised all colposcopic examinations. The Reid's Colposcopic Index (RCI)[11] was used to grade and classify the colposcopic findings. The RCI was used in order to standardise colposcopic interpretation and to minimise intra-observer variability.

The RCI considers four lesion signs characteristic of preinvasive cervical lesions: margin of the lesion, colour of the aceto-whitening, vessels within the lesion and iodine staining. The first three signs are evaluated following application of 5% acetic acid to the cervix. The final sign, iodine staining, is evaluated after the application of Lugol's iodine (25% strength) to the cervix.

The classification of the different colposcopic signs is illustrated in Table 5. Each sign is divided into 3 categories characteristic of various grades of disease and is assigned a numerical value from 0 – 2. The scores for each sign are combined to establish the total RCI score. A total RCI of 0 – 2 represents benign disease, HPV, or LSIL. A total score of 3- 5 represents CIN1 or CIN 2 (LSIL or HSIL) and 6-8 predicts CIN 2 or CIN 3 (HSIL).

The results of the colposcopic examination were entered into a coded data sheet (appendix B)

| Colposcopic Sign | 0 Points | 1 Point | 2 Points |
|---|---|---|---|
| Margin | Condylomatous or micropapillary contour<br>Indistinct borders<br>Flocculated or feathered margins<br>Jagged, angular lesions<br>Satellite lesions, acetowhitening that extends beyond TZ | Regular lesions with smooth outlines<br>Sharp peripheral margins | Rolled, peeling edges<br>Internal borders between areas of differing appearance |
| Colour | Shiny, snow-white colour<br>Indistinct aceto-whitening, semitransparent rather completely opaque | Shiny, off-white<br>Intermediate white | Dull, oyster grey |
| Vessels | Uniform, fine calibre<br>Randomly arranged with poorly formed patterns<br>Non-dilated capillary loops<br>Ill- defined areas of fine punctation or mosaic | Absence of surface vessels, following application acetic acid | Definite punctation or mosaicism<br>Individual vessels dilated, arranged in sharply demarcated, well-defined patterns |
| Iodine Staining | Positive iodine uptake, producing a mahogany-brown colour<br>Negative iodine uptake by an area that is recognisable as a low-grade lesion by above criteria (≤2/6) | Partial iodine uptake<br>Variegated, tortoise-shell appearance | Negative staining of a lesion, which is a high-grade lesion by the above criteria (≥ 3/6)<br>Mustard yellow appearance |
| Score | 0 – 2 benign or HPV or LSIL | 3 – 5 CIN 1 or CIN 2 (LSIL or HSIL) | 6 – 8 CIN 2 or 3 (HSIL) |

Table 5: Colposcopic signs used by the RCI for the grading of colposcopic findings[14]

### 2.5.2 Histological Sampling and Treatment

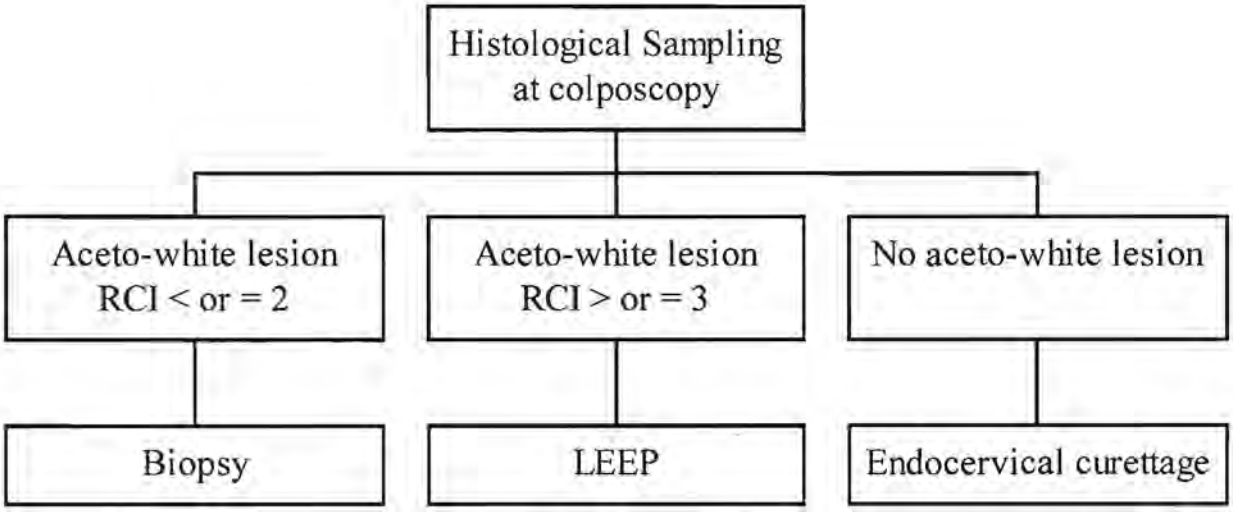
The protocol for histological sampling of the cervix is illustrated in Figure 12. If the total RCI was ≤ 2 a biopsy of the aceto-white lesion was performed. If no lesion was seen, endocervical curettage (ECC) was performed. ECC was introduced into the protocol after the first 280 women had been screened to ensure that occult endocervical disease would not be missed.

Women with an RCI of ≥ 3 were offered immediate treatment, on site, under local anaesthetic. Treatment was performed using the technique called Loop Electro-surgical Excision Procedure (LEEP), also known as Large Loop Excision of the Transformation Zone (LLETZ). LEEP or LLETZ surgically excises the entire transformation zone of the cervix to a depth of 7 – 10 mm using a thin wire electrosurgical electrode.

After colposcopic assessment and verbal consent from the patient, the cervix was injected with 2 to 6 mls of local anaesthetic (Prilocaine) containing a vasoconstrictive agent (vasopressin) [Octapressin™, Adcock Ingram]. Loop size was selected according to the size of the lesion. LEEP was performed using a Utah Finesse Electrosurgical Unit (Utah, Medical, USA). At the end of the procedure, the crater was electrocauterised. The amount of bleeding at the time of LEEP was documented and estimated as < 100 mls, > 100mls or none. In addition, women were asked whether they experienced pain, cramping, dizziness or faintness during the procedure and this was recorded on the colposcopy form (appendix B).

Women were informed about the symptoms and signs of the complications of LEEP (infection, haemorrhage and pain) and were asked to return should any complications have occurred. In addition, they were given a pre-printed letter explaining the procedure of LEEP and the possible complications, should it be necessary to consult after hours with other health care services. The author's emergency phone number was printed in the letter to facilitate communication with other health professionals.

Figure 11: Algorithm of colposcopic management



### 2.5.4 Special Circumstances

If the colposcopic assessment was inadequate due to the presence of infection or severe atrophy, women were treated with antibiotics or oestrogen, either orally or locally, and asked to return for a repeat colposcopic examination at a specified date.

If a significant aceto-white lesion was detected but the upper limit of the lesion was not seen or there was suspicion of microinvasion, a cone biopsy was performed either on site (loop excision conisation) or at Groote Schuur Hospital (cold-knife conisation). If an obvious cancer was found on examination, a biopsy was performed and the women were referred to Groote Schuur Hospital for further management.

### 2.5.5 Processing of Histological Specimens and Clinical Management

All histology specimens were placed in 10% formalin and saline and delivered to the UCT Department of Pathology on a weekly basis. The specimens were processed and evaluated at the UCT Department of Pathology, according to the routine protocol of the laboratory.

Clinical management of women was directed by the histological diagnosis determined by the UCT pathologists and women with a histological diagnosis of any grade of SIL were treated. Thus if a biopsy result on a woman with a RCI of $\leq 2$ or an ECC was called LSIL, HSIL or cancer, the patient was recalled for a repeat colposcopic examination and treatment.

### 2.5.6 Histological Review

Dr Thomas Wright, a principal co-investigator of the study, blindly reviewed all histology slides at the Department of Pathology, Columbia University, New York. For the purpose of analysis, the diagnosis made on blind histological review was used. If a woman had more than one histological specimen, the most severe diagnosis was used for analysis.

The histological diagnoses were classified as follows:

- Negative
- LSIL (CIN 1)
- HSIL (CIN 2 or 3)
- Malignant

The two-tiered system for the classification of cytological abnormalities, popularised by the Bethesda system, has not yet been widely adopted for histological reporting. A number of authors have however argued for this classification to be adopted for histological reporting. This is in recognition of the poor

inter and intra-observer reproducibility in distinguishing histologically between HPV infection only from CIN 1, CIN 1 from CIN 2 and CIN 2 from CIN 3[12]. In addition, low-grade lesions appear to represent a different clinical entity from high-grade lesions and in general, have a lower risk of progression to cancer. However, some low-grade lesions may progress to high-grade lesions in the absence of treatment.

For these reasons it was decided to classify the histological diagnoses in the study using the two-tiered system of LSIL and HSIL. Women with any grade of SIL were offered treatment to ensure that all cases of HSIL were identified.

## 2.6 Post-LEEP Follow-up

After treatment with LEEP, women were asked to return at 4 and 10 months post treatment for evaluation. At the post LEEP examination, women were interviewed about complications and whether they had returned to a clinic or hospital after treatment, and if so, for what reason and what type of treatment was given.

Thereafter a cervical smear was performed, followed by colposcopy utilising the RCI. If no lesion was seen an ECC was performed. All aceto-white lesions were biopsied regardless of the colposcopic grade and a second LEEP was performed only if the biopsy was positive for LSIL or HSIL.

The outcome of the post LEEP examination was entered into a coded data sheet. After the second post-LEEP examination women were asked to return to the study site for yearly cervical smears or were referred to the follow up cytology clinic at Groote Schuur Hospital and were no longer considered part of the study.

## 2.7 Tracing of Defaulters

The CHWs traced women who required colposcopy for any reason (positive screening test, positive biopsy, ECC or for a post-LEEP examination) and who did not return for examination, on a weekly basis. If after 4 successive visits a woman was not found or still did not return to the clinic for examination, she was no longer contacted

## 2.8 Ethics and Research Committee Approval

Approval for the study was obtained by the Ethics and Research Committee of the University of Cape Town and the Institutional Review Board of Columbia University, New York, NY.

## 2.9 Statistical Methods

All data were entered into coded data sheets and computerised at the Medical Research Council, Cape Town, South Africa. The data was analysed using SPSS statistical package.

The socio-demographic and clinical profile of the study population was described by calculating the proportions with specific characteristics for categorical variables (e.g. education, housing type etc.) and means (and/or medians) for continuous variables (e.g. age, parity etc.). All differences between proportions were tested using the Chi-Squared test and differences between means were tested using the t-test. Statistical significance was set at 0.05.

The prevalence of cervical disease was calculated as the proportion of women with complete follow-up information who had histologically confirmed low-grade SIL, high-grade SIL or invasive cancer (i.e. women scheduled for colposcopy but who were lost to follow-up were excluded from the denominators).

Risk factors for cervical disease were first considered in univariate analysis. In other words, we tested whether the prevalence of disease was different in women with the risk factor compared to women without the risk factor using the chi-squared test. Factors found to be statistically significant on the univariate analysis were then entered into a multivariate logistic regression analysis. In this model, the prevalence of cervical disease (outcome) was modelled as a logistic function of the relevant risk factors (predictors) simultaneously. Adjusted odds ratios (which provide a measure of the strength of the association between the risk factor and cervical disease adjusted for all the other risk factors in the model simultaneously) were calculated, as were 95% confidence intervals. Significance of individual risk factors was determined using the Wald test[13].

| | Gold standard 'Disease' | Gold standard 'No disease' | Total |
|---|---|---|---|
| Screening test positive | a | b | a+b |
| Screening test negative | c | d | c+d |
| Total | a+c | b+d | n |

Table 6: 2 x 2 Table for Calculation of Test Performance

To calculate sensitivity we defined the "gold standard" for disease to be histologically-confirmed low-grade SIL, high-grade SIL and invasive cancer. Sensitivity for detection of each of these categories individually, all three categories combined, and high-grade SIL and cancer alone were calculated. Sensitivity was defined as the proportion of women with the 'gold standard disease' who had a positive screening test or as a / (a+c) (definitions of positive screening tests are included in the description of the screening methods above) (Table 6). To calculate specificity we defined the "gold standard" for

'no disease' in two ways: (1) no positive screening tests or no disease of any grade after colposcopy and histological sampling or (2) no positive screening tests or no high-grade SIL or cancer after colposcopy and histological sampling (i.e. low-grade SIL was included as "no disease"). Specificity was defined as the proportion of women with the 'gold standard no disease' who had a negative screening test or as d / (b+d). We also calculated the positive predictive value as the proportion with disease among those with a positive screening test or as a / (a+b) and the negative predictive value as the proportion with no evidence of disease among those with a negative screening test or as d / (c+d). Again these calculations considered disease in combined and individual categories. 95% confidence intervals for sensitivity, specificity, and positive and negative predictive value were calculated using methods based on the binomial distribution for confidence intervals for proportions[14]. These calculations were restricted to women with satisfactory results for the relevant screening test and with complete follow-up data.

For the analysis of the sensitivity and specificity of HPV testing (both for the analysis of the Hybrid Capture 1 assay which was performed on all study subjects and for the analysis of the Hybrid Capture II assay which was performed on a specifically selected sub-sample of subjects) two different HPV DNA levels to define a 'positive' screening test were used. The first cut-off level classified only samples with relatively high levels of high-risk HPV DNA (RLU >10x positive control) as positive. The second cut-off classified samples with lower levels of HPV DNA (RLU >1x positive control) as being 'positive'. The second cut-off level is that defined by the manufacturers as the standard positive cut-off level. Since all HPV DNA measurements are quantitative, we also used Receiver Operating Characteristic (ROC) curves to investigate the consequences of shifting HPV DNA test cut-off values used to define a positive test.

An ROC curve plots sensitivity (y-axis) against 1-specificity (x-axis) obtained when cut-off values used to define a positive result on a test are shifted from the lowest through to the highest possible values. These curves can be used to identify optimal cut-off values which maximise both sensitivity and specificity simultaneously[18]. For these calculations, 'gold standard disease' was defined as histologically-confirmed high-grade SIL or cancer and 'gold standard no disease' was defined as no positive screening tests or no HSIL or cancer following colposcopy.

We also compared the quantities of HPV DNA detected using both Hybrid Capture 1 and Hybrid Capture II in women with and without disease. The mean $\log_{10}$ RLU values were tested using t-tests. Agreement between the two HPV tests was calculated using the kappa coefficient[15] (defining the results of each test dichotomously i.e. positive and negative) and using the Pearson correlation coefficient (using $\log_{10}$ RLU values).

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# Chapter 3
# Results

## 3.1 Study group, Recruitment, Socio-demographic Characteristics and Use of Contraception by the Study Sample

### 3.1.1 Study Group

From the middle of January 1996 to the end of September 1997, 2957 women were recruited to the study. Thirteen women were excluded from the analysis for the reasons set out in Table 7. The study group consisted of 2944 women.

| No. of women | Reasons for exclusion |
|---|---|
| 3 | Cervix inaccessible due to displacement of cervix by fibroids |
| 1 | Inability to visualise cervix due to gross obesity & vaginal wall prolapse |
| 1 | Previous cone biopsy |
| 1 | Fixed deformity of pelvis and patient unable to abduct her legs |
| 1 | Refused to be examined |
| 2 | Aged less than 35 years |
| 1 | Aborted 28 week fetus during examination |
| 3 | Temporarily excluded due to problems with electricity and never returned |

Table 7: Reasons for excluding 13 women

### 3.1.2 Recruitment

On average 150 women were recruited to the study per month. In June, August 1996 and February and March 1997, the number of women recruited dropped considerably. This was due to widespread violence in Site C caused by conflict between different taxi associations operating in the area. In September 1996, there was a delay in receiving equipment and supplies from the USA and the recruitment was stopped for 2 weeks. The project closed early in December 1996 as large numbers of women returned to the rural areas for the December holidays.

### 3.1.3 Socio-demographic Characteristics

The socio - demographic characteristics, reproductive and sexual histories and life-style habits of the study population are summarised in Table 8. The median age was 39 years. Most women (n = 1407) were in the 35 –39 year age group, followed by the 40 – 49 year age group (n = 1058), the 50 – 59 year age group (n = 389) and the smallest number of women were in the over 60 age group (n = 89). In one woman, the age was not recorded.

Although 93.4% of the women were born outside of Cape Town, 71.5% had been resident in Cape Town for over 5 years and 18.3% had lived in Cape Town for less than one year.

A considerable number of women had received no formal schooling (14.3%), less than half (42.6%) had some primary level schooling only, 38.8% had had some secondary level schooling and 3.6% of women had completed high school (matriculation). Less than half the women were married. About a third of the women were in formal employment and 70.5% of these women were employed in domestic service.

The majority of the women lived in serviced sites (62.7%) with approximately equal numbers of women living in 'unserviced sites' and brick houses. The majority of women (80%) living in serviced sites had electricity.

Most women had been pregnant with a median of 4 pregnancies (range 0 –14). Over a third of the women had more than 5 pregnancies and 3% of women were nulliparous. Similarly, median parity was 4 with over half the women having given birth to between 2 and 4 children. A third of women had given birth to 5 or more children. The median age of first pregnancy was 19 years (range 12 – 43 years). While 6.0% of women had their first pregnancy before 16 years of age, 40.4% of women had their first pregnancy as teenagers (aged 16 – 19 years).

The median age of first sexual experience was 17 years (range 10 – 32 years). While the first sexual experience was at less than 16 years of age in 17.2% of women, two thirds of women had their first sexual experience as teenagers (aged 16 – 19 years). The majority of women had between 2 and 5 lifetime partners with less than 3% of women reporting more than 5 lifetime partners. Just under a third of women reported being monogamous.

The majority of women (76.6%) were sexually active in the month prior to being interviewed. 8.6% of women used alcohol and of these, 62.5% reported excessive use. Smoking was reported by 7.6% of the women.

Some form of contraception had ever been used by 80.1% of women (Table 8). There were no data on 6 women. Of those women who had ever used contraception, the majority (90.7%) had used long acting intra-muscular progestogens (LAIP) and less than half of the ever users had used oral contraception (OC). Condom usage was rare, with 67 of the ever users of contraception having used condoms. Permanent contraception was a fairly common choice (28.6%). 'Other' types of contraception included intra-uterine devices, oral quinine tablets and a range of traditional herbs.

Current use of contraception (defined as use of contraception within the three months prior to being interviewed) was less common. Of the women who ever used contraception, 20.6% were currently using LAIP contraceptives and 2.9% of women were using OC. Only 10 women were currently using condoms.

| Variable | n* | % |
|---|---|---|
| Age (years) | | |
| 35 – 39 | 1407 | 47.8 |
| 40 – 49 | 1058 | 35.9 |
| 50 – 59 | 389 | 13.2 |
| 60 – 65 | 89 | 3.1 |
| Total | 2943 | |
| **Born in Cape Town** | | |
| Yes | 193 | 6.6 |
| No | 2750 | 93.4 |
| Total | 2943 | |
| Resident in Cape Town (years) | | |
| 0 – 1 | 501 | 18.3 |
| 1 – 5 | 279 | 10.2 |
| > 5 | 1956 | 71.5 |
| Total | 2736 | |
| Education | | |
| None | 420 | 14.3 |
| Primary only | 1254 | 42.6 |
| Some Secondary | 1142 | 38.8 |
| Matriculation | 106 | 3.7 |
| Tertiary | 19 | 0.6 |
| Total | 2941 | |
| Married | | |
| Yes | 1287 | 43.7 |
| No | 1655 | 56.2 |
| Total | 2942 | |
| Employed | | |
| Yes | 921 | 31.3 |
| No | 2023 | 68.7 |
| Type employment | | |
| Domestic service | 638 | 69.3 |
| Factory worker | 70 | 7.7 |
| Health worker | 32 | 3.5 |
| Traditional Healer | 5 | 0.5 |
| Other | 176 | 19.0 |
| Housing | | |
| Brick house with services | 546 | 18.6 |
| Serviced site | 1845 | 62.8 |
| Unserviced site | 548 | 18.6 |
| Total | 2939 | |
| Gravidity | | |
| 0 | 88 | 3.0 |
| 1 | 271 | 9.3 |
| 2 - 4 | 1471 | 50.0 |
| 5 or greater | 1111 | 37.7 |
| Total | 2941 | |
| Parity | | |
| 0 | 121 | 4.1 |
| 1 | 299 | 10.2 |
| 2 - 4 | 1523 | 51.8 |
| 5 or greater | 998 | 33.9 |
| Total | 2941 | |

| Variable | n | % |
|---|---|---|
| Age of first pregnancy (years) | | |
| < 16 | 170 | 6.0 |
| 16 – 19 | 1153 | 40.4 |
| 20 – 30 | 1504 | 52.7 |
| >30 | 26 | 0.9 |
| Total | 2853 | |
| Age first sex (years) | | |
| < 16 | 504 | 17.2 |
| 16 - 19 | 1947 | 66.2 |
| 20 – 32 | 487 | 16.6 |
| Total | 2938 | |
| Lifetime partners | | |
| Monogamous | 897 | 30.6 |
| 2 - 5 | 1952 | 66.6 |
| > 5 | 80 | 2.7 |
| Total | 2929 | |
| Sexual activity in month prior to interview | | |
| none | 680 | 23.2 |
| Yes | 2255 | 76.8 |
| Total | 2935 | |
| Alcohol Use | | |
| Excessive (4 or more drinks on one occasion) | 158 | 5.4 |
| Moderate (1 – 3 drinks on one occasion) | 95 | 3.2 |
| None | 2684 | 91.4 |
| Total | 2937 | |
| Smoking | | |
| Yes | 224 | 7.6 |
| No | 2715 | 92.4 |
| Total | 2939 | |
| Contraception use | | |
| Ever users | 2357 | 80.2 |
| Never users | 581 | 19.8 |
| Total | 2938 | |
| Type contraception used in women who ever used contraception | | |
| LAIP | 2138 | 90.7 |
| OC | 1060 | 44.8 |
| Sterilisation | 682 | 28.9 |
| Condoms | 67 | 2.9 |
| Other methods | 96 | 4.1 |
| Total | 2357 | |
| Contraception use in the last 3 months (% ever users) | | |
| LAIP | 487 | 20.7 |
| OC | 69 | 2.9 |
| Condoms | 10 | 0.4 |
| Other methods | 29 | 1.2 |
| Total | 595 | |

Table 8: Socio-demographic characteristics and contraception use among 2944 women recruited to the study (*Totals do not always add up to 2944 due to missing data)

### 3.1.4 Stratification of Socio-demographic Characteristics and Use of Contraception by Age

Stratifying the socio-demographic characteristics by age revealed that a greater proportion of the women aged 35 – 39 years had achieved matriculation or tertiary education, had had their first sexual experience before the age of 16 years and had more than 2 lifetime partners than women in the older age categories (Table 9). A greater proportion of the women over 50 years had no schooling and had more than five live births than women in the 35 – 39 age category.

More women in the 35 – 39 year age group were born in Cape Town (6.3%) than women in the over 60 age group (3.4%). The proportion of women in the different age groups who were employed was similar (between 30 and 34%) except in the over 60 group (4.5%). Just under 5% of the 35 – 39 year olds smoked compared to 9.0% of 40 – 49 year olds, 12.6% of 50 – 59 year olds and 13.5% of women over 60. The pattern of alcohol use was similar to smoking, with 7.8% of 35 – 39 year olds using alcohol compared to 11.6% of women over 50 years old.

Among women aged 35 – 39 years, 33.3 % of women had ever used OC, and 3.6% were currently using OC. By comparison, 55.4% of women in this age group had ever used LAIP and 25.9% were currently using LAIP. Among women in the 40 – 49 year age group 37.8% had ever used OC but only 1.7% were current users of the OC. In contrast, 65.0% of women in this age group had ever used LAIP and 11.2% were currently using LAIP.

| Variables | Age Groups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 35 – 39 yrs (n = 1407) | | 40 – 49 yrs ( n = 1058) | | 50 – 59 yrs (n = 389) | | 60 – 65 yrs (n = 89) | | Total (n = 2943) | |
| | n | % | n | % | n | % | n | % | n | % |
| Matric or 3° education | 99 | 7.0 | 24 | 2.3 | 2 | 0.5 | 0 | 0 | 125 | 4.2 |
| Age first sex <16 years | 296 | 21.0 | 170 | 16.1 | 35 | 9.0 | 4 | 4.5 | 505 | 17.2 |
| >2 lifetime partners | 604 | 42.9 | 407 | 38.3 | 93 | 23.9 | 9 | 10.1 | 1113 | 37.8 |
| Parity 2 – 4 | 881 | 62.6 | 492 | 46.5 | 125 | 32.1 | 25 | 28.1 | 1523 | 51.7 |
| Parity > or =5 | 213 | 15.1 | 476 | 45.0 | 250 | 64.3 | 59 | 66.3 | 998 | 33.9 |
| Ever users of OC | 468 | 33.2 | 400 | 37.8 | 111 | 28.5 | 13 | 14 | 992 | 33.7 |
| Ever users of LAIP | 780 | 55.4 | 688 | 65.0 | 174 | 44.7 | 17 | 19.1 | 1659 | 56.4 |

Table 9: Stratification of socio-demographic Characteristics and Use of Contraception by Age

## 3.2 Outcome of Clinical Examination, Screening Tests, Colposcopic Examination and Treatment of Women in the Study Sample

### 3.2.1 Clinical Examination

### 3.2.2 Visual Inspection of the Vulva and Vagina

Vulval lesions were noted in 7.6% (n = 223) of the women screened. The lesions were described as warty in 6.8% of women with vulvar lesions, ulcerative in 20.5%, inflammatory in 58.6% and as 'other' in 14.1%. A vaginal discharge was noted in 86.5% (n = 2544) of the women and this was classified as severe in 44.2% (n = 1124) of the cases.

### 3.2.3 Visual Inspection of the Cervix without Acetic Acid

The whole cervix was visualised in all but 20 women. Lesions were noted on the cervix with naked-eye examination (before application of acetic acid) in 10.6% (n = 313) of the women. The majority of these lesions were classified as inflammatory (84.7%), the rest being defined as exophytic (11.0%), endophytic (1.2%) and as an ulcer in one case.

Women with lesions on the cervix detected with naked-eye examination before the application of acetic acid were referred for colposcopy at the discretion of the nursing sister. For example, women with benign polyps or an inflamed cervix but no evidence of an aceto-white lesion were not referred for colposcopy. Of the 313 women with lesions noted on the cervix, 95 (30.4%) were also identified as DVI positive (i.e. an aceto-white lesion was noted after the application of acetic acid) and were referred for colposcopy.

### 3.2.4 Results of Screening Tests and Referral for Colposcopy:

The number of women with evaluable screening tests and the percentage with positive screening tests are shown in Table 10. All 2944 women underwent a DVI examination, one specimen for HPV DNA detection was unsatisfactory for testing, 22 (0.7%) cervical smears were unsatisfactory for evaluation and 333 (11.3%) of the Cervigrams™ were technically defective or unevaluable. Of the 333 Cervigrams™ that were not evaluable, 60% (n = 199) were unevaluable due to technical problems with the camera or with the electricity supply. A further 40% (n = 134) were unevaluable due to poor quality Cervigrams™ caused by the presence of blood, mucus or the speculum obscuring the cervix.

| Screening tests | Total No. Evaluable tests* | No. Positive (% Evaluable tests) | |
|---|---|---|---|
| | | n | % |
| DVI | | | |
| With naked-eye exam | 2944 | 534 | 18.1 |
| With magnification | 2944 | 534 | 18.1 |
| Cytology | 2922 | 238 | 8.1 |
| LSIL | | 155 | 5.3 |
| HSIL | | 80 | 2.7 |
| Cancer | | 3 | 0.1 |
| Cervicography™ | 2611 | 276 | 10.6 |
| 'Warrants Colposcopy' | | 35 | 1.3 |
| LSIL | | 203 | 7.7 |
| HSIL | | 29 | 1.1 |
| Cancer | | 9 | 0.3 |
| HPV DNA (10x) | 2943 | 180 | 6.1 |
| HPV DNA (1x) | 2943 | 465 | 16.2 |

Table 10: Number of unsatisfactory screening tests, number of evaluable screening tests and the number of woman who required referral for colposcopy. [No. = number. * The total number of evaluable tests does not add up to 2944 due to unevaluable or unsatisfactory tests]

Of the 2944 women screened, 18.1% (n = 534) were considered DVI positive and referred for colposcopy. Visualisation of the cervix with the monocular lens did not detect any aceto-white lesions that were not detected by unmagnified examination of the cervix.

A positive cervical smear (LSIL, HSIL or cancer) was diagnosed in 8.1% (n = 238) of women with satisfactory smears. A positive Cervigram™ (warrants colposcopy, LSIL, HSIL or cancer) was diagnosed in 10.6 % (n = 276) of women with evaluable Cervigrams™. HPV DNA at 10 times the positive control was detected in 6.1% (n = 180) and HPV DNA at 1x the positive control was detected in 16.2 % (n = 465) of the women screened.

Of women with evaluable cervical smears 84.8% (n = 2478) were normal and 87.3% (n = 2279) of evaluable Cervigrams™ were evaluated as normal. A diagnosis of ASCUS was made in 7.0% (n = 206) of cervical smears compared to 2.1% (n = 56) of Cervigrams™ that were evaluated as 'atypical'. While cytological evaluation reported HSIL in 2.7% of cases, the Cervicography™ evaluation was HSIL in 1.1%. Cytology reported LSIL in 5.3% of smears compared to 7.7% of Cervigram™ evaluations of LSIL. Cancer was reported in 3 cervical smears and 9 cervigrams.

### 3.2.5 Colposcopic Examination

In total, 28.7% (n = 842) of women were positive on any of the four screening tests and were referred for colposcopy. Of these, 91.0% (n = 765) were examined colposcopically. The findings at colposcopy are illustrated in the algorithm in Figure 13.

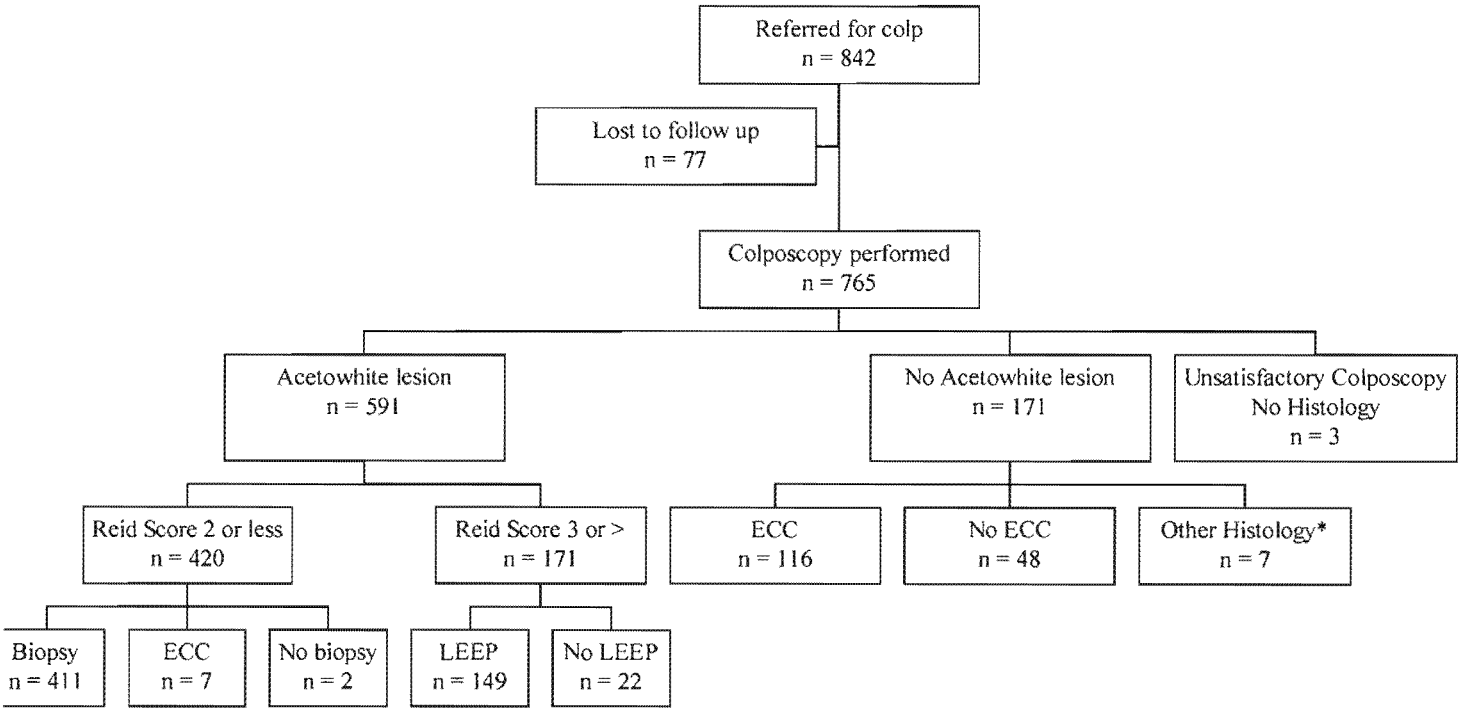



Figure 12: Algorithm of women referred to colposcopy and the outcome of the colposcopic examination [* Other histology: 4 women had biopsies, one had a hysterectomy for a prolapsed fibroid, one had a cone biopsy and one had a polypectomy]

Aceto-white lesions were seen in 77.3% (n = 591) of the women who underwent colposcopy. Of these women, 420 had a RCI ≤ 2 and a biopsy was performed in 411 cases. An ECC was performed in 7 women with a RCI ≤ 2 because the aceto-white lesion was located in the endocervix and was technically difficult to biopsy. In two cases, the colposcopic assessment of the cervix was normal and no histological sampling was performed. These women were called negative for disease.

A RCI of ≥ 3 was diagnosed in 171 of the women who had aceto-white lesions at colposcopy and a LEEP was performed in 87.1% (n = 149) of these women. Despite a Reid score of ≥ 3, the remaining 22 women did not have a LEEP performed for the following reasons:

- 9 women had an obvious cancer and a biopsy was performed
- 4 women were found to be pregnant and a biopsy was performed on one of them
- 1 woman refused the procedure

- 3 women required cone biopsies for suspected micro-invasion or upper limit of the lesion not seen
- 1 woman required a cone biopsy but this was technically impossible and a hysterectomy was performed
- 1 woman required treatment for severe vaginitis and did not return for a repeat colposcopy
- In 3 cases the RCI was ≥ 3 but the biopsy did not confirm the presence of a lesion (in all cases immediate LEEP was not performed due to technical difficulties)

The colposcopic assessment was inadequate in a further three women and it was impossible to assign a Reid Score and no histological sampling was performed. These women were treated and asked to return for repeat colposcopy, but all three defaulted.

No aceto-white lesion was noted at colposcopy in 171 women. No ECC was performed in 50 women. Of these 50 women, ECC was not performed in 48 women as ECC was not performed at the beginning of the study. One woman had a large prolapsed fibroid and had a hysterectomy and no CIN was found in the hysterectomy specimen. One woman had an endocervical polypectomy only. These 50 women were coded as negative for disease for the purpose of analysis. An ECC was performed in the remaining 121 (67.8%) women who had no visible lesion at colposcopy.

In addition to the 149 LEEPs performed on a 'see and treat' basis, a further 29 LEEPs were performed. These 29 women were recalled for repeat colposcopy despite a RCI < 3 on the basis of the diagnosis of LSIL or HSIL made by the UCT pathologists (n = 28) and a discrepancy between positive screening tests and colposcopy (n = 1).

In summary, there were a total of 178 LEEPS performed: 149 were performed on a 'see and treat' basis, 28 after repeat colposcopy and prior histological sampling, and one after repeat colposcopy because of a discrepancy between positive screening tests and the original negative colposcopy.

### 3.2.6 Histological Diagnosis after LEEP performed on a 'See and Treat' basis

Of the 149 women who underwent LEEP on a 'see and treat' basis, there were 45 (30.2%) cases of histologically-confirmed LSIL, 42 (28.2%) cases of HSIL and one case of micro-invasion. In 61 (40.9%) of the cases there was no SIL or cancer detected in the LEEP specimen.

### 3.2.7 Women with Positive Screening Tests who did not undergo Colposcopy

Of the 842 women referred for colposcopy, 9.1% (n = 77) did not receive a colposcopic examination. The majority of these women either gave incorrect addresses or did not live at the addresses they gave, or they had returned to the rural areas and were no longer resident in Cape Town.

The number of women with positive screening tests who were not examined colposcopically is summarised in Table 11. Overall, 4.5% of the women who were DVI positive, 5% of the women who were HPV DNA positive (at the 10x level), 14.3 % of the women with positive cervical smears and 13.4 % of the women with positive Cervigrams™ were not examined colposcopically.

| Screening test | Total No. Positive | No. of colposcopies performed | | No. Lost to Follow Up | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| DVI | 534 | 510 | 95.5 | 24 | 4.5 |
| Cytology | 238 | 204 | 85.7 | 34 | 14.3 |
| Cervicography™ | 276 | 239 | 86.5 | 37 | 13.4 |
| HPV DNA (10x) | 180 | 171 | 95.0 | 9 | 5.0 |

Table 11: Total number of women with positive screening tests who underwent colposcopy and who were lost to follow up [No. = number]

Analysed in more detail, a trend of increasing lost to follow up over time emerges. For instance, there were 100 women who were DVI and HPV DNA negative but who had a positive cervical smear and 24% of these women did not undergo a colposcopic examination. Similarly, there were 90 women who were DVI negative, HPV DNA negative and had a negative cervical smear but had a positive Cervigram™ and 29% of these women were not successfully traced for colposcopy.

### 3.2.8 Final Disease Status of the Study Sample

The final disease status of the 2944 women included in the study is as follows: 71.4% (n = 2102) were negative on all four screening tests and were considered by definition as having no SIL or cancer. After colposcopic examination and histological sampling, there was no disease diagnosed in a further 19.7% (n = 579) women. A histological diagnosis of LSIL was made in 3.2% (n = 95) of women, HSIL in 2.5% (n = 74) and cancer in 0.4% (n = 12) of women. No diagnosis was made in 2.8% (n = 82) women who were lost to follow up or did not undergo histological sampling. These results are summarised in Table 12.

| Final disease status of study sample | n | % |
|---|---|---|
| Negative all four screening tests | 2102 | 71.4 |
| No SIL or cancer after histological sampling | 579 | 19.7 |
| LSIL | 95 | 3.2 |
| HSIL | 74 | 2.4 |
| Cancer | 12 | 0.4 |
| Lost to follow up* | 82 | 2.6 |
| Total | 2944 | 100 |

Table 1: Final disease status of the 2944 women recruited to the study [*includes 4 women who underwent colposcopy but no histological sampling was performed and one woman who had a biopsy that was unsatisfactory for histological sampling]

The majority of the women with cancer (n = 7) had advanced disease (stage IIb and above) and all these women were symptomatic. One of the women with Stage Ib and the two women with stage Ia were asymptomatic. There were 2 women with FIGO stage Ia, 3 with stage Ib, 3 with stage II b, 3 with stage III b and one with stage IVa.

### 3.2.9 Distribution of SIL and Cancer in 5 year age groups

The proportion of women in five-year age categories who had histologically confirmed LSIL, HSIL or cancer is shown in Table 13. There was a tendency for a decreased prevalence of LSIL in women over 50 years compared to younger women. HSIL was diagnosed in 4.5% of women over 60 years compared to 3.0% of women in the 35 – 39 age group, but this difference was not statistically significant. There were no cancers in the 35 – 39 year age group compared to 3.4% of the over 60 age group and this different was highly significant.

The prevalence of disease in the different age groups using smoothed 10-year age intervals is illustrated in Figure 13. The same pattern of disease prevalence is seen in this graph as is seen using 5-year age intervals as shown in Table 13. Of note in Figure 13, is the much higher prevalence of HPV DNA positivity (1x) compared to the prevalence of SIL or cancer.

| Histological diagnosis | Age categories in years | | | | | | | | | | | | | | P Value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 35 – 39 (n = 1365) | | 40 – 44 (n = 602) | | 45 – 49 (n = 429) | | 50 – 54 (n = 221) | | 55 – 59 (n = 156) | | 60 – 65 (n = 87) | | Total (n = 2860*) | | |
| | n | % | n | % | n | % | n | % | n | % | n | % | n | % | |
| LSIL | 51 | 3.7 | 27 | 4.5 | 10 | 2.3 | 2 | 0.9 | 4 | 2.6 | 1 | 1.1 | 95 | 3.3 | 0.07 |
| HSIL | 42 | 3.1 | 14 | 2.3 | 8 | 1.9 | 3 | 1.4 | 3 | 1.9 | 4 | 4.5 | 74 | 2.6 | 0.38 |
| Cancer | 0 | 0 | 4 | 0.7 | 3 | 0.7 | 0 | 0 | 2 | 1.3 | 3 | 3.4 | 12 | 0.4 | **<0.000** |

Table 13: Proportion of women in each age category who had histologically confirmed LSIL, HSIL or cancer [* excludes women who were lost to follow up]

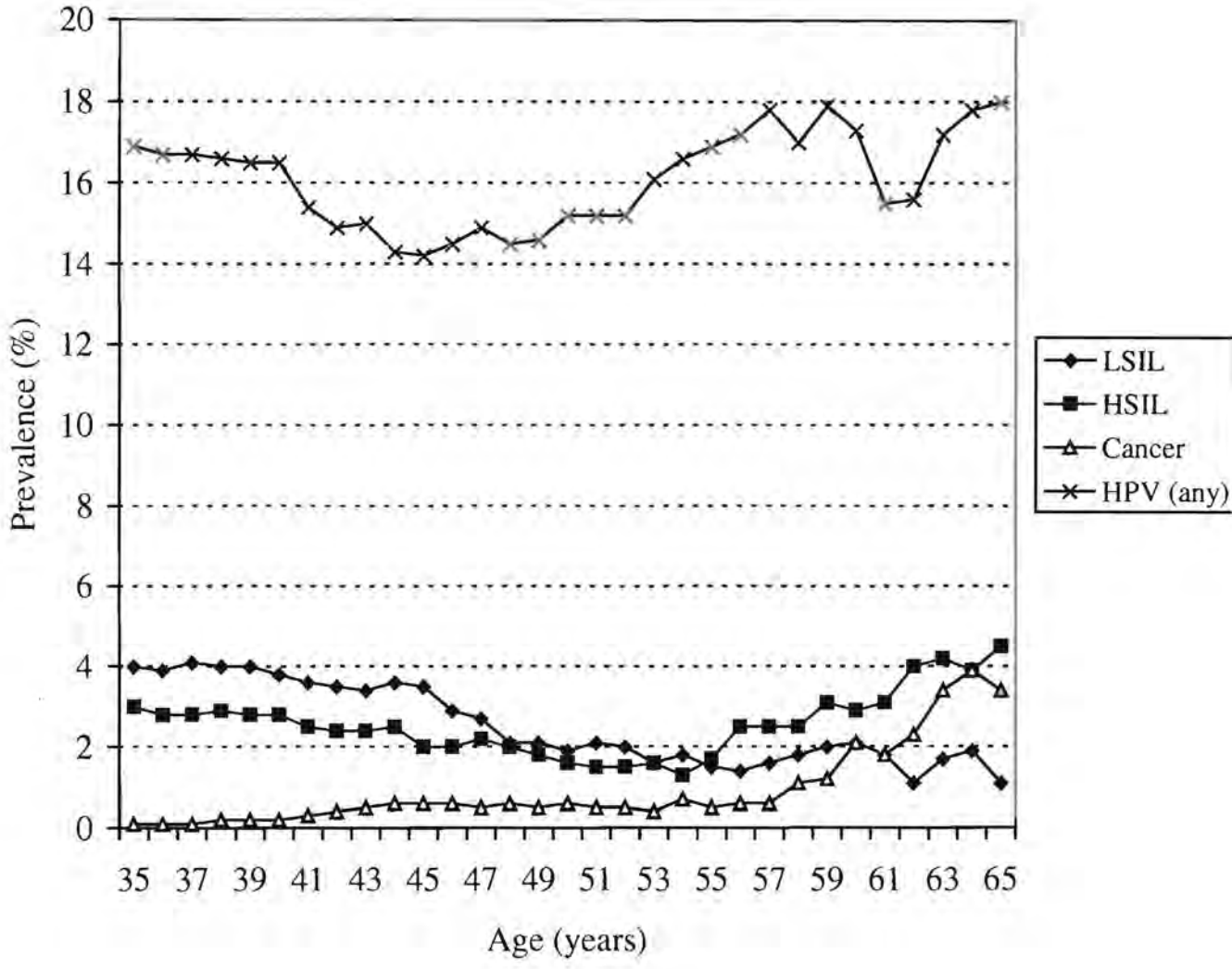



Figure 13: Prevalence of HPV DNA, LSIL, HSIL and cancer using smoothed 10 year age intervals

## 3.3 Socio-demographic Factors in relation to HSIL and Cancer, Women Lost to Follow-up and a Positive HPV DNA Test (1x)

### 3.3.1 Association of Socio-demographic Factors with HSIL and Cancer

The results of the univariate analysis for the risk of having HSIL or cancer are shown in Table 14. Smoking, current use of LAIP and not being married were associated with HSIL. Increasing age and use of alcohol were the only risk factors significantly associated with cancer.

Multivariate logistic regression analysis was used to calculate the odds ratios (OR) and 95% confidence intervals (CI) for the various risk factors. All factors that were significant on univariate analysis were put into the multivariate analysis. For HSIL, the association with being unmarried was increased, but not significantly with an OR of 1.55 (95% CI: 0.95 – 2.56). The association with smoking was no longer significant with an OR of 1.97 (95% CI: 0.99 – 3.93). The association with use of LAIP remained significant with OR of 2.17 (95% CI: 1.29 – 3.64). The association between HSIL and the use of LAIP specifically controlling for age, smoking and being currently sexually active was 2.08 (95% CI: 1.21 – 3.58).

For cancer, the association with each year of increased age remained significant with an OR of 1.12 (95% CI: 1.06 – 1.20) but the association with alcohol was no longer statistically significant (OR = 2.78; 95% CI: 0.72 – 10.65).

| Variable | Total No. women | HSIL | | P value | Cancer | | P value |
|---|---|---|---|---|---|---|---|
| Age | | n | % | | n | % | |
| 30 – 39 | 1365 | 42 | 3.1 | | 0 | 0 | |
| 40 – 49 | 1031 | 22 | 2.1 | | 7 | 0.7 | |
| 50 – 59 | 377 | 6 | 1.6 | | 2 | 0.5 | |
| 60 – 65 | 88 | 4 | 4.5 | 0.17 | 3 | 3.4 | **<0.000** |
| Education | | n | % | | n | % | |
| No school | 407 | 13 | 3.2 | | 2 | 0.5 | |
| Primary | 1213 | 29 | 2.4 | | 6 | 0.5 | |
| Some secondary | 1116 | 29 | 2.6 | | 4 | 0.4 | |
| Matric or tertiary | 123 | 3 | 2.4 | 0.85 | 0 | 0 | 0.84 |
| Born in Cape Town | | n | % | | n | % | |
| Yes | 193 | 8 | 4.1 | | 0 | 0 | |
| No | 2668 | 66 | 2.5 | 0.16 | 12 | 0.4 | 0.35 |
| Age first sexual intercourse | | n | % | | n | % | |
| < 16 | 486 | 15 | 3.1 | | 1 | 0.2 | |
| > or = 16 | 2371 | 59 | 2.5 | 0.12 | 11 | 0.5 | 0.42 |
| Married | | n | % | | n | % | |
| Yes | 1254 | 24 | 1.9 | | 4 | 0.3 | |
| No | 1606 | 50 | 3.1 | **0.05** | 8 | 0.5 | 0.46 |
| Parity | | n | % | | n | % | |
| Nulliparous | 115 | 1 | 0.9 | | 0 | 0 | |
| One | 293 | 7 | 2.4 | | 1 | 0.3 | |
| 2 – 4 | 1482 | 43 | 2.9 | | 3 | 0.2 | |
| 5 or > | 969 | 23 | 2.4 | 0.54 | 8 | 0.8 | 0.11 |
| No. of sexual partners | | n | % | | n | % | |
| 0 – 2 | 1773 | 45 | 2.5 | | 8 | 0.5 | |
| 3 or more | 1076 | 29 | 2.7 | 0.80 | 4 | 0.4 | 0.75 |
| Smoking | | n | % | | n | % | |
| Yes | 216 | 10 | 4.6 | | 2 | 0.9 | |
| No | 2641 | 63 | 2.3 | **0.04** | 10 | 0.4 | 0.23 |
| Alcohol use | | n | % | | n | % | |
| Yes | 240 | 10 | 4.2 | | 3 | 1.3 | |
| No | 2615 | 64 | 2.4 | 0.11 | 9 | 0.3 | **0.04** |
| OC | | n | % | | n | % | |
| Never | 1827 | 55 | 3.0 | | 8 | 0.4 | |
| Ever | 966 | 17 | 1.8 | | 4 | 0.4 | |
| Current | | 2 | 2.9 | 0.06 | 0 | 0 | 0.64 |
| LAIP | | n | % | | n | % | |
| Never | 750 | 15 | 2.0 | | 5 | 0.6 | |
| Ever | 1615 | 37 | 2.3 | | 7 | 0.4 | |
| Current | 471 | 22 | 4.7 | **0.02** | 0 | 0 | 0.36 |

Table 14: Univariate analysis of risk factors for HSIL and cancer [OC = Oral contraception; LAIP = Long acting injectable progestogens]

### 3.3.2 Association of Socio-demographic Factors with Women who were Lost to Follow-up

A univariate analysis of the association between socio-demographic factors and women who failed to return for their colposcopic examination was performed. Of the women who required colposcopy and who were born in Cape Town, 100% (56/56) were followed up as opposed to 90.2% (709/786) of the women who were not born in Cape Town ($p = 0.007$). In addition, women who lived in unserviced sites ($p = 0.03$), who used alcohol ($p = 0.03$) and were nulliparous ($p = 0.01$) were significantly less likely to return for follow up. Age, marital status, employment, level of education, age of first sexual intercourse, smoking and use of contraception were not significantly associated with follow up.

### 3.3.3 Association of Socio-demographic Factors with a Positive HPV DNA test (1x)

Table 15 shows the prevalence of HPV DNA (1x) in relation to socio-demographic characteristics with the unadjusted prevalence ratios. The prevalence of HPV DNA was lowest in women aged 40 – 49 years (13.8%). Prevalence of HPV DNA was higher in women who lived in unserviced sites, were unmarried, had more than two sexual partners during their lifetime, had at least one live birth and were current users of LAIP. The association between HPV DNA and current use of LAIP was reduced (OR 1.2 95% CI: 0.85 –1.69) after adjusting for age, education, housing type, marital status, current sexual activity, lifetime number of partners, age of first sexual intercourse, parity and cigarette smoking. The association between HPV DNA prevalence and current sexual activity was somewhat stronger (OR 1.4 95% CI: 1.07 – 1.84) after adjusting for the same variables. All other associations were similar in univariate and multivariate analysis, as were associations using higher levels of HPV DNA (10x) as the outcome.

| Variable | n | Prevalence HPV (%) | Unadjusted Prevalence Ratio (95% CI) |
|---|---|---|---|
| Age (years) | | | |
| 35 – 39 | 1407 | 17.5 | |
| 40 – 49 | 1058 | 13.8 | 0.79 (0.65 – 0.95) |
| 50 – 59 | 389 | 17.2 | 0.99 (0.77 – 1.26) |
| 60 - 65 | 89 | 18.0 | 1.03 (0.65 – 1.63) |
| Education | | | |
| None | 419 | 18.6 | |
| Some Primary | 1254 | 15.6 | 0.84 (0.66 – 1.06) |
| Some Secondary | 1142 | 16.0 | 0.86 (0.68 – 1.09) |
| Matric or tertiary | 125 | 14.4 | 0.77 (0.48 – 1.24) |
| Housing | | | |
| Brick House | 546 | 13.0 | |
| Serviced Site | 1844 | 16.3 | 1.26 (0.99 – 1.60) |
| Unserviced Site | 548 | 18.6 | 1.43 (1.08 – 1.88) |
| Married | | | |
| Yes | 1286 | 12.8 | |
| No | 1655 | 18.7 | 0.69 (0.58 – 0.82) |
| Currently sexually active | | | |
| Yes | 2260 | 16.4 | |
| No | 680 | 15.3 | 1.07 (0.88 – 1.31) |
| >2 sexual partners | | | |
| Yes | 1113 | 18.1 | |
| No | 1816 | 14.9 | 1.21 (1.02 – 1.43) |
| Age first sex < 16 years | | | |
| Yes | 505 | 17.4 | |
| No | 2433 | 15.8 | 1.10 (0.89 – 1.36) |
| Parity | | | |
| Nulliparous | 121 | 7.4 | |
| One | 298 | 15.4 | 0.48 (0.24 - 0.95) |
| 2 – 4 | 1523 | 16.8 | 1.09 (0.82 – 1.45) |
| 5 or more | 998 | 16.4 | 1.06 (0.79 – 1.44) |
| LAIP Use | | | |
| Never | 794 | 15.0 | |
| Ever | 1659 | 15.4 | 1.03 (0.84 – 1.25) |
| Current | 487 | 20.3 | 1.36 (1.07 – 1.73) |
| Smoking | | | |
| Yes | 224 | 16.1 | |
| No | 2714 | 16.1 | 1.00 (0.73- 1.36) |

Table 15: Prevalence of high risk HPV DNA (1x) among 2943 women by socio-demographic and clinical characteristics and use of contraception

## 3.4 Performance of the Screening Tests

### 3.4.1 Results of the Screening Tests among the 2944 Women Screened and the Histological Diagnosis in Women who underwent Colposcopy

The results of the four screening tests among the 2944 women screened and the histological diagnosis in women who underwent colposcopy is shown in Table 16. The data on women who were HPV DNA positive at the 1x cut-off are shown for purposes of comparison.

Women who were negative on all four screening tests were not referred for colposcopy for reasons explained in the methods. The prevalence of disease in the individual negative screening tests is estimated from the colposcopic examination of women who were negative on one screening test, but positive on one or more of the other screening tests. This represents the number of known cases of disease 'missed' by the individual negative screening tests.

| Screening test | Result of screening test | No. of women (%) | | No. of women who underwent colposcopy | | No. of women (%) with each histological diagnosis who underwent colposcopy | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | No disease n = 579 | | LSIL n = 95 | | HSIL n = 74 | | Cancer n = 12 | |
| | | n | % | n | % | n | % | n | % | n | % | n | % |
| DVI | Negative | 2410 | 81.9 | 250 | 10.4 | 174 | 69.9 | 48 | 19.2 | 25 | 10.0 | 3 | 1.2 |
| | Positive | 534 | 18.1 | 510 | 95.5 | 405 | 79.4 | 47 | 9.2 | 49 | 9.6 | 9 | 1.8 |
| Cytology | WNL | 2478 | 84.8 | 505 | 20.4 | 455 | 90.1 | 34 | 6.7 | 16 | 3.2 | 0 | 0.0 |
| | ASCUS | 206 | 7.0 | 43 | 20.9 | 34 | 79.1 | 7 | 16.3 | 2 | 4.7 | 0 | 0.0 |
| | LSIL | 155 | 5.3 | 128 | 82.6 | 64 | 50.0 | 43 | 33.6 | 20 | 15.6 | 1 | 0.8 |
| | HSIL | 80 | 2.7 | 73 | 91.3 | 21 | 28.8 | 11 | 15.1 | 35 | 47.9 | 6 | 8.2 |
| | Cancer | 3 | 0.1 | 3 | 100 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 100 |
| HPV DNA | <1 | 2467 | 83.8 | 492 | 19.9 | 436 | 88.6 | 33 | 6.7 | 21 | 4.3 | 2 | 0.4 |
| | 1 - 10 | 296 | 10.1 | 97 | 32.8 | 59 | 60.8 | 18 | 18.6 | 15 | 15.5 | 5 | 5.2 |
| | >10 | 180 | 6.1 | 171 | 95.5 | 84 | 49.1 | 44 | 25.7 | 38 | 22.2 | 5 | 2.9 |
| CG™ | Negative | 2279 | 87.3 | 427 | 18.7 | 346 | 81.0 | 49 | 11.5 | 30 | 0.7 | 2 | 0.5 |
| | Atypical | 56 | 2.1 | 28 | 18.7 | 24 | 85.7 | 3 | 10.7 | 1 | 3.6 | 0 | 0.0 |
| | WC | 35 | 1.3 | 29 | 82.9 | 21 | 72.4 | 2 | 6.9 | 6 | 20.7 | 0 | 0.0 |
| | LSIL | 203 | 7.8 | 173 | 85.2 | 124 | 71.7 | 26 | 15.0 | 23 | 13.3 | 0 | 0.0 |
| | HSIL | 29 | 1.1 | 28 | 96.6 | 8 | 28.6 | 10 | 35.7 | 9 | 32.1 | 1 | 3.6 |
| | Cancer | 9 | 0.3 | 9 | 100 | 2 | 22.2 | 0 | 0.0 | 0 | 0.0 | 7 | 77.8 |

Table 16: Results of the four screening tests among 2944 women screened and the histological diagnosis among those who underwent colposcopy [* Columns do not add up to 2944 because women with unsatisfactory smears, one missing HPV DNA result and defective Cervigrams™ are excluded. The difference in the number of women with positive screening tests and the number of women who underwent colposcopy, represents women who were lost to follow up or women who had negative screening test but underwent colposcopy due to one or more other positive screening tests]

Of the 12 women with invasive cancer and 74 with histologically confirmed HSIL identified in the study, DVI correctly identified 75% (n = 9) of the cancers and 63.5 % (n = 47) of the HSIL lesions. Similarly, cytology identified 83.3% (n = 10) of the cancers and 75.3% (n = 55) of the cases of HSIL. HPV DNA testing at the 10x level identified 33.3% (n = 5) of the cancers and 51.3% (n = 38) of the cases of HSIL compared to 83.3% (n = 10) of the cancers and 71.6% (n = 53) of the cases of HSIL if

the lower cut-off was used. Cervicography™ identified 66.7% (n = 8) of the cancers and 51.3% (n = 38) of the cases of HSIL.

No disease was found after colposcopic assessment and histological sampling in 79.4% of women with a positive DVI test, compared to 41.7% of women who had a positive cervical smear, 64.8 % of women with a positive Cervigram™ and 49.1% of women with a positive HPV DNA test (10x). Of the women with a positive HPV DNA test at the lower threshold (1x) and who underwent colposcopy because of having other positive screening tests, 72.3% had no disease confirmed histologically.

There were 22 women who had unsatisfactory smears and 8 women had a colposcopic examination on the basis of other positive screening tests: one woman had HSIL (12.5%), two had cancer (25%) and 5 women had no disease (62.5%). Of the 333 women with unevaluable Cervigrams™, 66 had a colposcopic examination on the basis of other positive screening tests: 5 women had LSIL (7.6%), 5 had HSIL (7.6%), 2 had cancer (3.0%) and 54 women had no disease (81.8%).

### 3.4.2 Sensitivity and Specificity of the Screening Tests

As outlined in the methods section, the sensitivity and specificity of each screening test was calculated assuming that women who were negative on all four screening tests and women who had colposcopy but negative histology, were negative for SIL or cancer. Disease positive women were those who underwent colposcopy and after histological sampling had a diagnosis of LSIL, HSIL or cancer or HSIL or cancer. Women who were lost to follow up or who had unevaluable screening tests were excluded from the calculations for test performance.

The estimated sensitivities, specificities, and 95% confidence intervals of the different screening tests for any disease, HSIL, HSIL and cancer are shown in Table 17.

| Screening tests | Sensitivity - any disease | Sensitivity - HSIL | Sensitivity - HSIL + Cancer | Specificity - no disease | Specificity - HSIL + Ca |
|---|---|---|---|---|---|
| DVI | 58.0 (50.5 – 65.2) | 66.2 (54.2 – 76.6) | 67.4 (56.4 – 76.9) | 84.9 (83.5 – 86.2) | 83.7 (82.3 – 85.1) |
| Cytology | 66.9 (59.4 – 73.6) | 75.3 (63.6 – 84.4) | 78.3 (67.7 – 86.3) | 96.8 (96.1 – 97.4) | 95.0 (94.0 – 96.0) |
| CG™ | 50.3 (42.6 – 58.1) | 50.8 (38.0 – 63.5) | 58.2 (46.6 – 69.1) | 93.5 (92.4 – 94.4) | 92.2 (91.1 – 93.2) |
| HPV (10x ) | 48.1 (40.6 – 55.6) | 51.4 (39.5 – 63.0) | 50.0 (39.1 – 60.9) | 96.9 (96.1 – 97.5) | 95.4 (95.5 – 96.1) |
| HPV (1x) | 69.1 (61.7 – 75.6) | 71.6 (59.8 – 81.2) | 73.3 (62.4 – 82.0) | 87.8 (86.5 – 89.0) | 89.2 (88.0 – 90.4) |

Table 17: Sensitivities and specificities of the screening tests - 95% confidence intervals in brackets [any disease = LSIL, HSIL or cancer, no disease = no LSIL, HSIL or cancer, CG™ = Cervicography; HPV (10x = HPV DNA positive at 10 x the positive control; HPV (1x = HPV DNA positive at any level positive]

Comparing the sensitivities of the screening tests for any disease, cytology and HPV DNA testing (1x) performed equivalently and DVI performed marginally better than Cervicography™ or HPV DNA testing (10x). DVI, cytology and HPV DNA testing (1x) had similar sensitivities for the detection of HSIL and were higher than the sensitivities of either Cervicography™ or HPV DNA testing (10x).

For HSIL and cancer, the sensitivity of DVI was marginally lower than the sensitivities of cytology and HPV DNA testing (1x). Cervicography™ and HPV DNA testing at the 10x level had lower sensitivities for HSIL and cancer than the other screening tests and were equivalent to one another.

DVI had the lowest specificity for HSIL and cancer at 83.7. Cytology and HPV DNA testing at the 10x level had the highest specificity and the specificity of Cervicography™ was lower than the specificity of cytology. While lowering the threshold for HPV DNA detection to any level positive would have increased the sensitivity of the test, the specificity decreased to 89.2.

### 3.4.3 Estimation of Sensitivity and Specificity using Cytology as the 'gold standard'

In an attempt to estimate the degree of verification bias introduced into our calculations of sensitivity and specificity by the failure to apply the 'gold standard' (i.e. histology) to all women who were screened, we estimated the sensitivity and specificity of the screening tests using cytology as the 'gold standard' (a test that was applied to all women who were screened). Specifically we estimated the sensitivity of the tests for HSIL and cancer where women with a normal smear, ASCUS or LSIL were considered 'negative for disease'. Women with a smear of HSIL or cancer were considered 'positive for disease'. The results of this analysis are shown in Table 18.

| Screening test | Sensitivity (HSIL and cancer) | Specificity (HSIL and Cancer) |
|---|---|---|
| DVI | 53.0 (41.8 – 63.9) | 83.0 (81.6 – 84.4) |
| HPV DNA (1x) HC I | 77.1 (66.3 – 85.3) | 85.6 (84.2 – 86.8) |
| HPV DNA (1x) HC II | 80.7 (75.5 – 85.1) | 64.8 (59.7 – 69.7) |
| Cervicography™ | 50.0 (38.6 – 61.4) | 90.7 (89.5 – 91.8) |

Table 18: Sensitivity and Specificity of the Screening tests using Cytology as the 'Gold Standard'

While the sensitivity of DVI for HSIL and cancer using cytology as the gold standard is somewhat lower than when histology is used as the gold standard, the specificity is unaltered. The sensitivity and specificity of HPV DNA testing at the 1x level using HC I and of Cervicography™ remain unchanged. The specificity of HPV DNA testing using HC II is significantly lower, however.

### 3.4.4 Positive and Negative Predictive Values of the Screening Tests

The PPVs of each screening test and the 95% confidence intervals are shown in Table 19. DVI and HPV DNA testing (1x) had the lowest PPVs compared to the other screening tests. Cytology and HPV DNA testing (10x) had the highest PPVs and were equivalent to one another. Cervicography™ had a PPV intermediate between DVI and cytology. Using the lower threshold for HPV DNA detection reduced the PPV of HPV DNA testing for HSIL and cancer by nearly 50%.

| Screening test | PPV - any disease | PPV - HSIL and cancer |
|---|---|---|
| DVI | 20.5 (17.2 – 24.4) | 11.4 (8.8 – 14.5) |
| Cytology | 58.3 (51.2 – 65.1) | 31.9 (25.6 – 38.8) |
| Cervicography™ | 35.1 (29.2 – 41.6) | 19.2 (14.6 – 24.9) |
| HPV (10x) | 50.8 (43.2 – 58.6) | 25.0 (19.0 – 32.5) |
| HPV (1x) | 27.7 (23.7 – 32.1) | 14.0 (11.0 – 17.6) |

Table 19: PPVs of the screening tests for any disease (LSIL, HSIL and cancer and HSIL and cancer with the 95% Confidence Intervals in brackets

The NPVs of the screening tests are shown in Table 20. Cytology and HPV DNA testing (1x) had the highest NPV for HSIL and cancer, with DVI, Cervicography™ and HPV DNA testing (10x) performing equally.

| Screening test | NPV - no disease | NPV - HSIL and cancer |
|---|---|---|
| DVI | 96.8<br>(96.0 – 97.4) | 98.8<br>(98.5 – 99.4) |
| Cytology | 97.8<br>(97.1 – 98.3) | 99.3<br>(97.8 – 98.8) |
| Cervicography | 96.3<br>(95.7 – 97.2) | 98.6<br>(98.0 – 99.0) |
| HPV (10x) | 96.5<br>(95.7 – 97.2) | 98.4<br>(98.9 – 99.6) |
| HPV (1x) | 97.7<br>(97.0 – 98.2) | 99.0<br>(98.3 – 99.2) |

Table 20: NPVs of the screening tests for no disease and for HSIL and cancer with the 95% Confidence Intervals in brackets

## 3.5 Factors Influencing the Performance of the Screening Tests

### 3.5.1 Sensitivity, Specificity, PPV and NPV of the Screening Tests in Three Age Categories

The sensitivities and specificities of the screening tests for HSIL and cancer in three age groups are shown in Table 21. While the sensitivity of DVI in the 40 – 49 year age groups appears higher than the other age groups this difference is not statistically significant (p = 0.22). Similarly, Cervicography™ appeared to perform better in the 40 – 49 year age group (p = 0.34). Cytology had the highest sensitivity in the over 50 age group (p = 0.79). In contrast, HPV DNA testing at both the 10x and 1x levels appeared to perform slightly worse in the 40 – 49 year age group compared to younger and older age groups (p = 0.27 and 0.17 respectively).

The specificity of DVI in the older age group (> 50 years) was significantly greater than in younger age groups, as was the specificity of cervicography, both visual screening techniques. The specificities of the other screening tests did not vary in the three age groups.

| Screening test + Age in years | Sensitivity | P value | Specificity | P value |
|---|---|---|---|---|
| DVI | | | | |
| 35 – 39 | 59.5 | | 83.1 | |
| 40 – 49 | 79.3 | | 82.1 | |
| 50 - 65 | 66.7 | 0.22 | 88.9 | **0.004** |
| Cytology | | | | |
| 35 – 39 | 78.6 | | 95.0 | |
| 40 – 49 | 75.0 | | 94.0 | |
| 50 - 65 | 84.6 | 0.79 | 95.6 | 0.56 |
| CG™ | | | | |
| 35 – 39 | 53.8 | | 90.4 | |
| 40 – 49 | 70.4 | | 93.2 | |
| 50 - 65 | 46.2 | 0.34 | 95.0 | **0.005** |
| HPV (10x) | | | | |
| 35 – 39 | 57.1 | | 94.7 | |
| 40 – 49 | 37.9 | | 96.2 | |
| 50 - 65 | 53.3 | 0.27 | 95.6 | 0.31 |
| HPV (1x) | | | | |
| 35 – 39 | 81.0 | | 85.0 | |
| 40 – 49 | 58.6 | | 87.9 | |
| 50 - 65 | 80.0 | 0.17 | 84.9 | 0.10 |

Table 21: Sensitivities, specificities and P values for HSIL and cancer of the different screening tests in three age categories

The PPVs of the screening tests for HSIL and cancer in the three age groups are shown in Table 22. The PPVs of all the screening tests appear to increase with age, but the numbers of women with HSIL or cancer in the older age groups are small and the observed differences are not statistically significant.

| Screening test | PPV | P Value | NPV | P Value |
|---|---|---|---|---|
| DVI | | | | |
| 35 – 39 | 10.1 | | 98.5 | |
| 40 – 49 | 11.4 | | 99.3 | |
| 50 – 65 | 16.4 | 0.35 | 98.8 | 0.28 |
| Cytology | | | | |
| 35 – 39 | 34.4 | | 99.3 | |
| 40 – 49 | 32.5 | | 99.3 | |
| 50 – 65 | 35.5 | 0.94 | 99.5 | 0.84 |
| CG ™ | | | | |
| 35 – 39 | 15.4 | | 98.4 | |
| 40 – 49 | 24.0 | | 99.0 | |
| 50 - 65 | 24.0 | 0.23 | 98.1 | 0.36 |
| HPV (10x) | | | | |
| 35 - 39 | 25.5 | | 98.6 | |
| 40 – 49 | 22.4 | | 98.2 | |
| 50 - 65 | 28.6 | 0.83 | 98.4 | 0.74 |
| HPV (1x) | | | | |
| 35 - 39 | 14.7 | | 99.3 | |
| 40 – 49 | 12.3 | | 98.7 | |
| 50 - 65 | 15.0 | 0.79 | 99.2 | 0.32 |

Table 22: PPVs, NPVs and P values of the screening tests for HSIL and cancer in the three age categories

Sensitivities and specificities of the different screening tests for HSIL and cancer using smoothed 10-year age groups are illustrated graphically in Figures 14 - 17

Figure 14: Sensitivity and specificity of cytology for HSIL and cancer by age

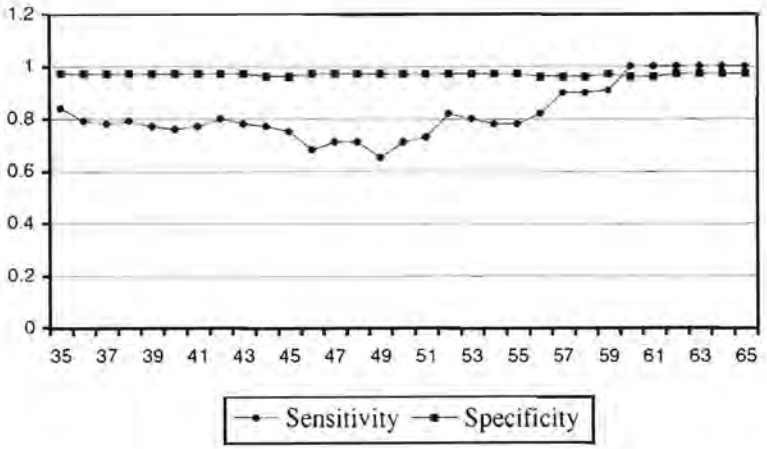



Figure 15: Sensitivity and specificity of Cervicography™ for HSIL and cancer by age

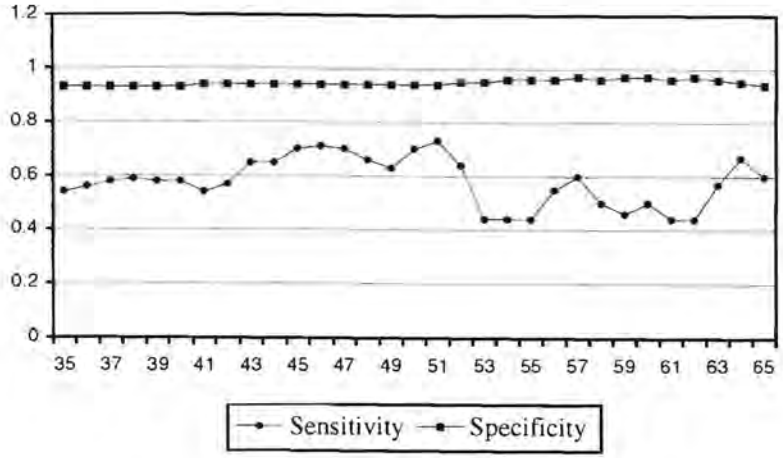



Figure 16: Sensitivity and specifity of DVI for HSIL and cancer by age

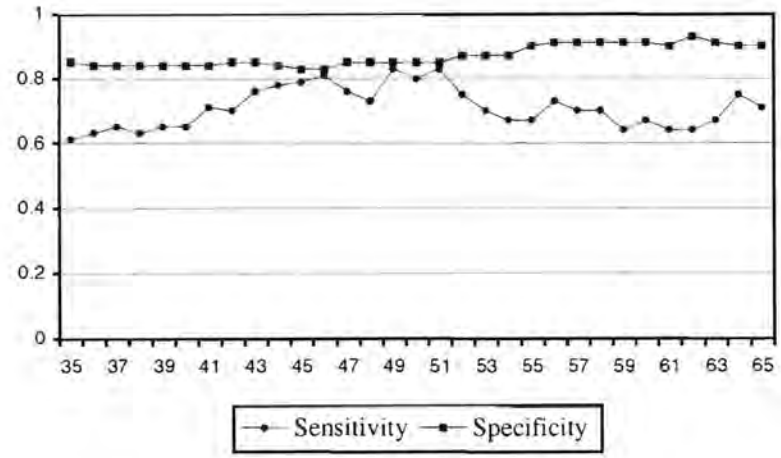
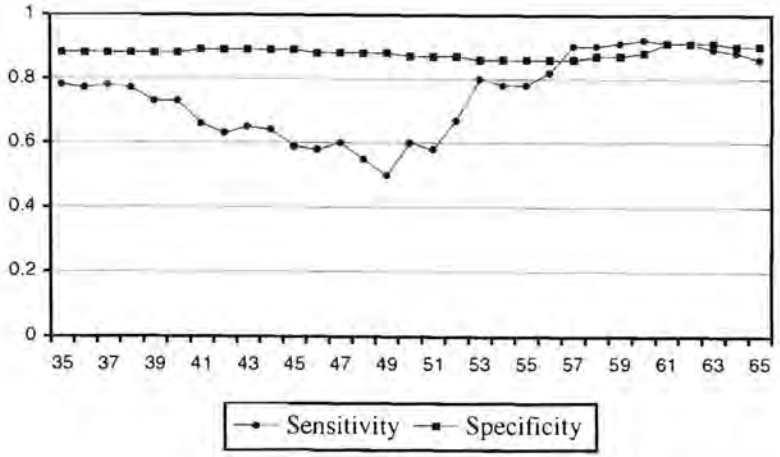



Figure 17: Sensitivity and specificity of HPV DNA testing (1x) for HSIL and cancer



### 3.5.2 Other Factors Influencing Performance of the Different Screening Tests

The sensitivity of DVI was much higher among women with cervices that were described as inflamed by the nursing sister, compared to women whose cervices were not described as inflamed (92.3 % compared to 63.0%; p = 0.04) but there was corresponding decrease in specificity (74.9 versus 84.5% respectively; p = 0.000).

There was no statistical difference in sensitivity and specificity of the screening tests among women who used LAIP, nor was there a difference in relation to parity. Due to very small numbers of women who were current users of OC, it was not possible to meaningfully calculate the influence of OC use on the performance of the screening tests

## 3.6 Number of cases of LSIL, HSIL and Cancer Detected with One, Two, Three or Four Positive Screening Tests

Table 23 shows the number of cases of LSIL, HSIL and cancer that were detected in women with one, two, three or four positive screening tests and who underwent colposcopy.

Of the 29 women who were positive on all four screening tests and who underwent colposcopy, 55.2% had HSIL and 6.9% had cancer compared to 30.9% HSIL and 10.9% cancer in the 55 women who were positive on three screening tests and underwent colposcopy. Of the 169 women who were positive on two screening tests and who underwent colposcopy, 14.2 % had HSIL and 1.2% had cancer compared to 3.3 % HSIL and 0.4% cancer in women with one screening test positive.

Of the 318 women who had a positive DVI and who underwent colposcopy but were negative on the three other screening tests, 3.4 % had HSIL or cancer compared to 11% of women who were DVI positive in combination with other positive screening tests. Of the women who were negative on all screening tests except cytology, 7.7% had HSIL (there were no cancers) compared to 32% of women overall who were positive on cytology and other combinations of screening tests.

Similarly, of women who had a positive Cervigram™ only, 1.5% had HSIL (there were no cancers) compared to 23% of women who had a positive Cervigram and other screening tests. Of women who were positive on HPV DNA testing (10x) only, 3.4% had HSIL compared 25% of women with a positive HPV DNA test (10x) in combination with other screening tests.

Of the women who had only two screening tests positive, the greatest proportion of HSIL or cancer was detected by the combinations of positive cytology and a positive HPV DNA test (10x) and positive cytology and a positive Cervigram™ (30.8 and 30.0% respectively). Of the women with the combination of positive cytology and a positive DVI test, HSIL or cancer was diagnosed in 20%

compared to 8.3% of women who were DVI positive and HPV DNA positive (10x) or 6.7% of women who were DVI positive and Cervigram™ positive.

| Screening tests | | | | | No. (% of women with disease who underwent colposcopy | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DVI | HPV | Pap | CG | Total | | | | | | | |
| - | - | - | - | 2102 | | | | | | | |
| One screening test positive | | | | | | | | | | | |
| | | | | | | LSIL | | HSIL | | Cancer | |
| DVI | HPV (10x) | Pap | CG | Test Pos | No. colps* | n | % | n | % | n | % |
| - | - | - | + | 90 | 65 | 3 | 4.6 | 1 | 1.5 | 0 | 0 |
| - | - | + | - | 86 | 65 | 13 | 20.0 | 5 | 7.7 | 0 | 0 |
| - | + | - | - | 60 | 59 | 10 | 16.9 | 2 | 3.4 | 0 | 0 |
| + | - | - | - | 337 | 318 | 13 | 4.1 | 9 | 2.8 | 2 | 0.6 |
| Total | | | | 573 | 507 | 39 | 7.7 | 17 | 3.3 | 2 | 0.4 |
| Two screening tests positive | | | | | | | | | | | |
| DVI | HPV (10x) | Pap | CG | Test Pos | No. colps* | n | % | n | % | n | % |
| + | + | - | - | 12 | 12 | 3 | 25.0 | 1 | 8.3 | 0 | 0 |
| + | - | + | - | 15 | 15 | 0 | 0 | 3 | 20.0 | 0 | 0 |
| - | - | + | + | 14 | 10 | 5 | 50.0 | 3 | 30.0 | 0 | 0 |
| - | + | - | + | 4 | 3 | 1 | 33.3 | 0 | 0 | 0 | 0 |
| - | + | + | - | 44 | 39 | 12 | 30.8 | 11 | 2.6 | 2 | 5.1 |
| + | - | - | + | 93 | 90 | 11 | 12.2 | 6 | 6.7 | 0 | 0 |
| Total | | | | 182 | 169 | 32 | 18.9 | 24 | 14.2 | 2 | 1.2 |
| Three screening tests positive | | | | | | | | | | | |
| DVI | HPV (10x) | Pap | CG | Test Pos | No. colps* | n | % | n | % | n | % |
| + | + | + | - | 12 | 12 | 6 | 50.0 | 5 | 41.7 | 0 | 0 |
| - | + | + | + | 10 | 9 | 4 | 44.4 | 3 | 33.3 | 1 | 11.1 |
| + | - | + | + | 27 | 25 | 6 | 24.0 | 9 | 36.0 | 5 | 20.0 |
| + | + | - | + | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | | 57 | 55 | 16 | 29.0 | 17 | 30.9 | 6 | 10.9 |
| Four screening tests positive | | | | | | | | | | | |
| DVI | HPV (10x) | Pap | CG | Test Pos | No. colps* | n | % | n | % | n | % |
| + | + | + | + | 30 | 29 | 8 | 27.6 | 16 | 55.2 | 2 | 6.9 |
| Total | | | | 842 | 760 | 95 | 12.5 | 74 | 9.7 | 12 | 1.6 |

Table 23: Number of cases of LSIL, HSIL and cancer detected with one, two, three or four positive screening tests [* The difference between the number of positive cases and the number of colposcopies performed, represents the number of women lost to follow up; + = screening test positive; - = screening test negative; Test pos. = total number of cases that were screening test positive; No. colps = number of colposcopies performed; CG™ = Cervicography™]

## 3.7 Evaluation of the Performance of Colposcopy in the Triage of Women with Positive Screening Tests

Colposcopy is traditionally accepted to be an essential step towards a definitive diagnosis of positive screening tests, such as an abnormal cervical smear. Colposcopy is used to direct appropriate histological sampling of the cervix, to exclude occult micro-invasive disease and to determine which aceto-white lesions represent significant pathology and require treatment.

Colposcopic assessment identified 170 of the women who underwent colposcopy as having SIL or cancer (i.e. RCI ≥ 3). Of these women, 6.5% had cancer, 25.9% had HSIL, 26.5% had LSIL and 41.2% had no disease confirmed histologically (Table 23). Of the 417 women classified as having no clinically significant disease at colposcopy (i.e. RCI = 1 – 2), 6.2% had HSIL and 11.3% had LSIL confirmed histologically (there were no cancers). When expressed in terms of what proportion of women with SIL or cancer were miss-classified as lacking significant disease by a colposcopic assessment of RCI of 1 – 2, colposcopy miss-classified 49.5% (47/95 of the cases of LSIL) and 35.1% (26/74 of the cases of HSIL).

In contrast, of the 173 women with no aceto-white lesion identified at colposcopy, one woman with micro-invasive cancer, 2.3% with HSIL and 1.7% with LSIL were miss-classified as having no disease (Table 24).

| Colposcopic diagnosis | Histological diagnosis after colposcopic evaluation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No disease | | LSIL | | HSIL | | Cancer | |
| | n | % | n | % | n | % | n | % |
| RCI ≥ 3 (n = 170) | 70 | 41.2 | 45 | 26.5 | 44 | 25.9 | 11 | 6.5 |
| RCI < 1 - 2 (n = 417) | 344 | 82.5 | 47 | 11.3 | 26 | 6.2 | 0 | 0 |
| No aceto-white (n = 173) | 165 | 95.4 | 3 | 1.7 | 4 | 2.3 | 1 | 0.6 |
| Total (n = 760) | 579 | 76.2 | 95 | 12.5 | 74 | 9.7 | 12 | 1.6 |

Table 24: Number of women with no disease, LSIL, HSIL or cancer who had a colposcopic evaluation of RCI ≥ 3, RCI < 1 -2 and no aceto-white lesion seen at colposcopy

The type of positive screening test influenced the sensitivity and specificity of colposcopy for HSIL and cancer (Table 25). For instance, the sensitivity of a colposcopic diagnosis of RCI ≥ 3 for HSIL and cancer in women who were DVI positive was 58.6% compared to a sensitivity of 79% when the referring screening test was a positive cervical smear, 75.6% for a positive HPV DNA test (10x) and 70% for a positive Cervigram™. Of note however, the specificity of DVI for HSIL and cancer was increased by the combination of DVI and a colposcopic assessment of RCI ≥ 3 whereas it was decreased for the other screening tests.

| Positive Screening tests | Colposcopic assessment RCI ≥ 3 | |
|---|---|---|
| | Sensitivity HSIL + Cancer | Specificity HSIL + Cancer |
| DVI | 58.6 | 87.6 |
| Cytology | 79.0 | 66.0 |
| Cervicography™ | 70.0 | 70.0 |
| HPV DNA (10x) | 75.6 | 69.5 |

Table 25: Sensitivity and specificity of a Colposcopic Assessment of RCI ≥ 3 for HSIL and cancer

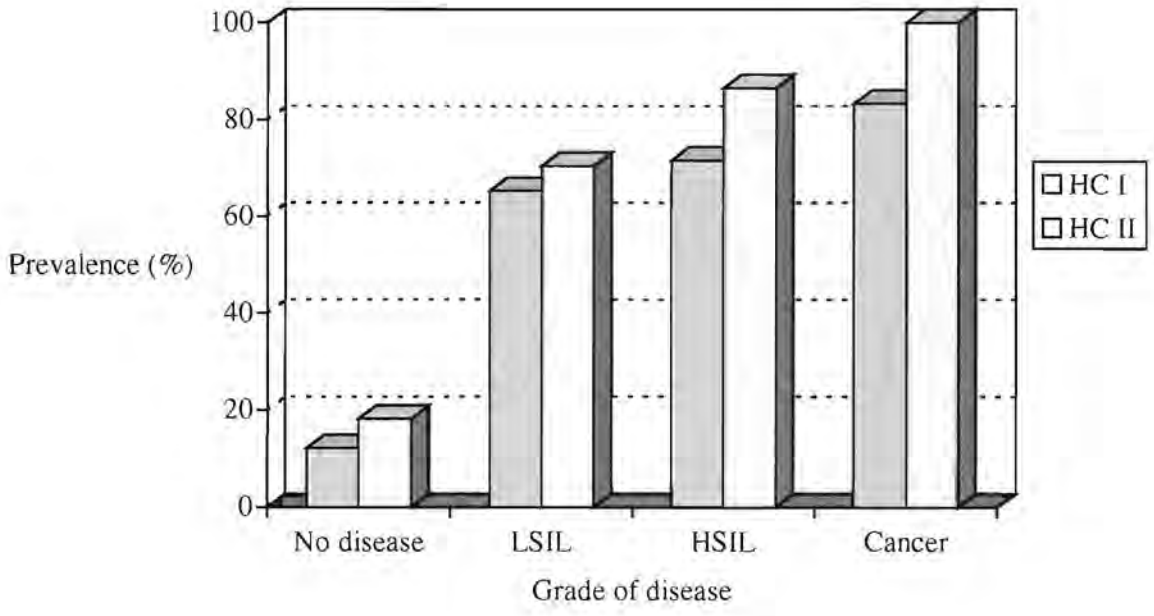
## 3.8 HPV DNA testing

### 3.8.1 Comparison of HPV DNA Testing using Hybrid Capture I and Hybrid Capture II in relation to Disease Detection

As mentioned in the methods section a sample of stored HPV DNA specimen collection tubes were retested at Columbia University using the newer generation Hybrid Capture Assay, known as HC II (Digene Diagnostics, Sliver Spring, MD). The sample consisted of all women with histologically confirmed LSIL, HSIL or cancer and a 10% random sample (n = 243) of women with no evidence of disease (either all screening tests negative or no evidence of disease after colposcopy and histological sampling). This section presents the results of the HC II test and compares them to the HCI (tube-based assay) that was used to screen women in the study.

The difference in the prevalence of HPV DNA positivity in the different grades of disease, using HC I (tube-based test) and HC II (micro-titre plate test), is illustrated in Figure 18.

Figure 18: Graphic illustration in the prevalence of no disease, SIL and cancer using the two different Hybrid Capture tests



HPV DNA testing (1x) using HC I was positive in 12.2% of women with no disease, 65.3% (62/95) of cases of LSIL, 71.6% (53/74) of HSIL, 83.3% (10/12) of cancers. Compared to women who were negative on all four screening tests or who had no disease confirmed histologically, the prevalence of HPV DNA was 6.8-fold higher (95% CI: 5.20 – 8.97) in women with cancer, 5.9-fold higher (95% CI: 4.93 – 7.00) in women with HSIL, and 5.4-fold higher (95% CI: 4.48 – 6.40) in women with LSIL.

Using HC II, HPV DNA prevalence in all disease categories was higher than HC I. HPV DNA using HC II was positive in 18.2% (44/243) of women with no disease, 70.5% (67/95) of women with LSIL, 86.5% (64/74) of HSIL and 100% (12/12) of women with cancer. The prevalence of HPV DNA in women with histologically confirmed disease was roughly equivalent between HC II using a 10x cut-off and HC I using a 1x cut-off.

Overall, among women with HSIL, 10.8% were missed by both HC I and HC II, 2.7% were detected by HC I but not HC II, and 17.6% were detected by HC II and not HC I. Among women with no evidence of disease, 9.5% were classified as positive by both tests, 4.5% were classified as positive by HC I and not HC II, and 8.6% were classified as positive by HC II and not HC I. Kappa coefficients indicated good agreement stratified by disease category: 0.41 among women with HSIL, 0.54 among women with LSIL and 0.51 among with no disease or negative screening tests.

The sensitivities and specificities of HPV DNA testing using Hybrid Capture I and II at different cut-offs for a positive test are shown in Table 26. The sensitivity of the HC II test for HSIL and for cancer is considerably higher than the sensitivity of HC I. However the high sensitivity of the test is at the expense of a lower specificity.

| Type of HPV test | Sensitivity | | | Specificity |
|---|---|---|---|---|
| | LSIL (n = 95) | HSIL (n =74) | Cancer (n = 12) | No disease (n = 2681) |
| **HC I** | | | | |
| HPV >1x pos control | 65 | 72 | 83 | 88 |
| HPV > 10x pos control | 46 | 51 | 42 | 97 |
| **HC II** | | | | Random sample (n = 243) |
| Microtitre plate test | | | | |
| HPV > 1x pos control | 70 | 86 | 100 | 82 |
| HPV >10x pos control | 59 | 74 | 100 | 90 |

Table 26: Sensitivity and specificity of HC I and HC II for SIL and cancer

Quantities of HPV DNA measured using HC I and HC II were significantly correlated (r = 0.81) although mean levels of HPV DNA were approximately 10-fold higher using HC II (Figure 21). Agreement between the two tests was good (kappa = 0.67) if both tests were classified as positive to 1 x the positive control. Agreement was marginally better (kappa = 0.77) if HC I was classified as positive (1x) and HC II as 10x the positive control.

Although HPV DNA prevalence in both tests increased with the grade of disease, the quantity of HPV DNA detected in positive samples did not vary significantly between the different grades of disease. Using HC I and restricting the analysis to women with detectable HPV DNA, the geometric mean levels (± standard deviation) of HPV DNA were 11.4 ± 6.61 in women with cancer, 21.8 ± 3.88 in women with HSIL, and 21.7 ± 4.86 in women with LSIL. Using HC II, geometric mean levels were 113.8 ± 6.92 in women with cancer, 149.3 ± 7.90 in women with HSIL and 150.0 ± 8.78 in women with LSIL (Figure 19).

### 3.8.2 Estimated ROC Curves

ROC curves comparing the relative performance of HC I and HC II are shown in Figure 20. The area under the ROC curve was 0.88 for HC II and 0.83 for HC I, indicating slightly better performance of HC II. However, when the cut – off values of the two tests were set such the prevalence of HPV DNA was below 8%, the corresponding abilities of the two tests to identify confirmed cases of HSIL or cancer were almost identical. HC II had improved detection of cases of HSIL and cancer compared to HC I only once higher prevalence (i.e. lower cut-off values) was allowed (Figure 20).

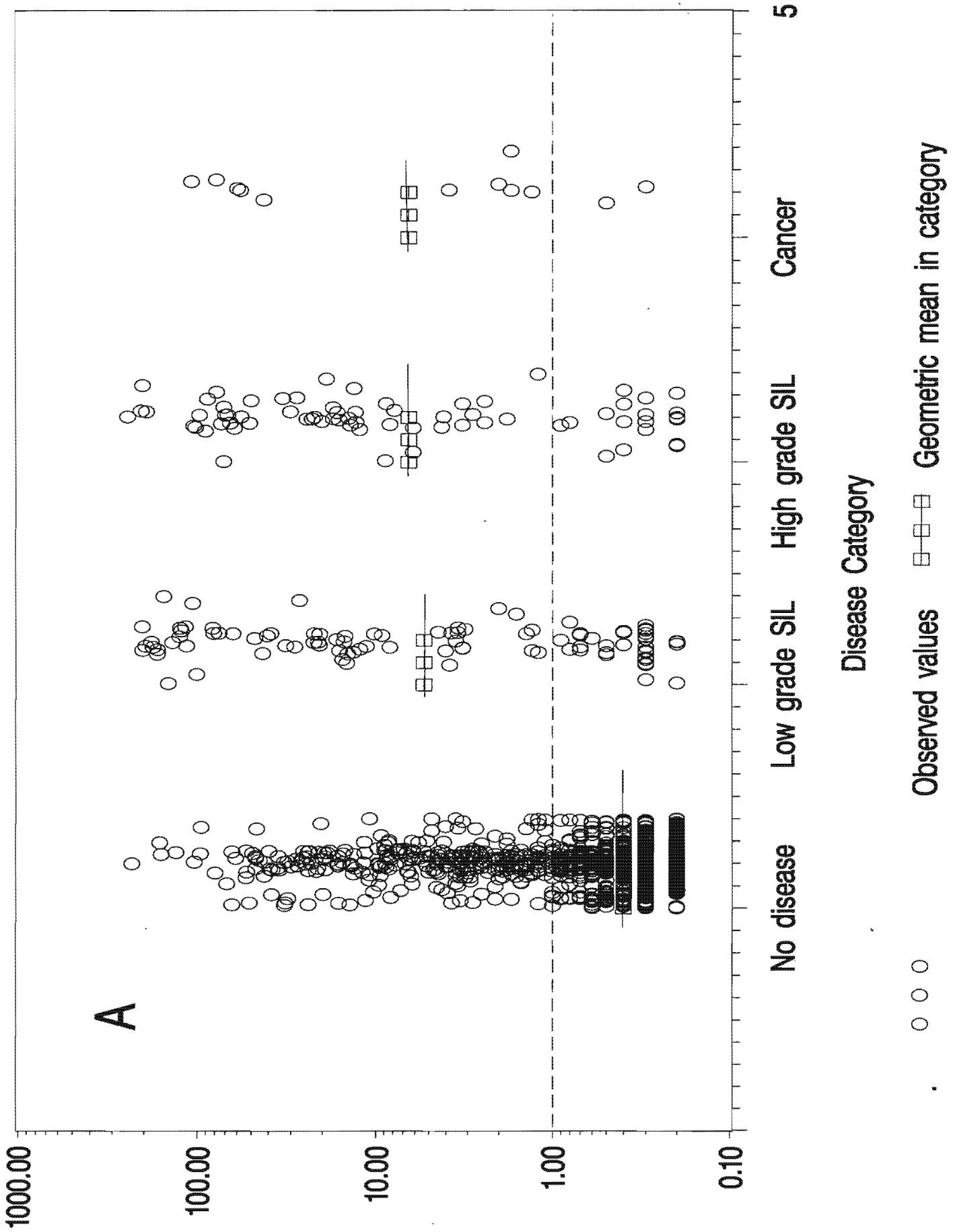
### 3.8.3 Proportion of HSIL and Cancer Classified as HPV DNA Positive using HC I and HC II with Different Cut-off Values to Define a Positive Test

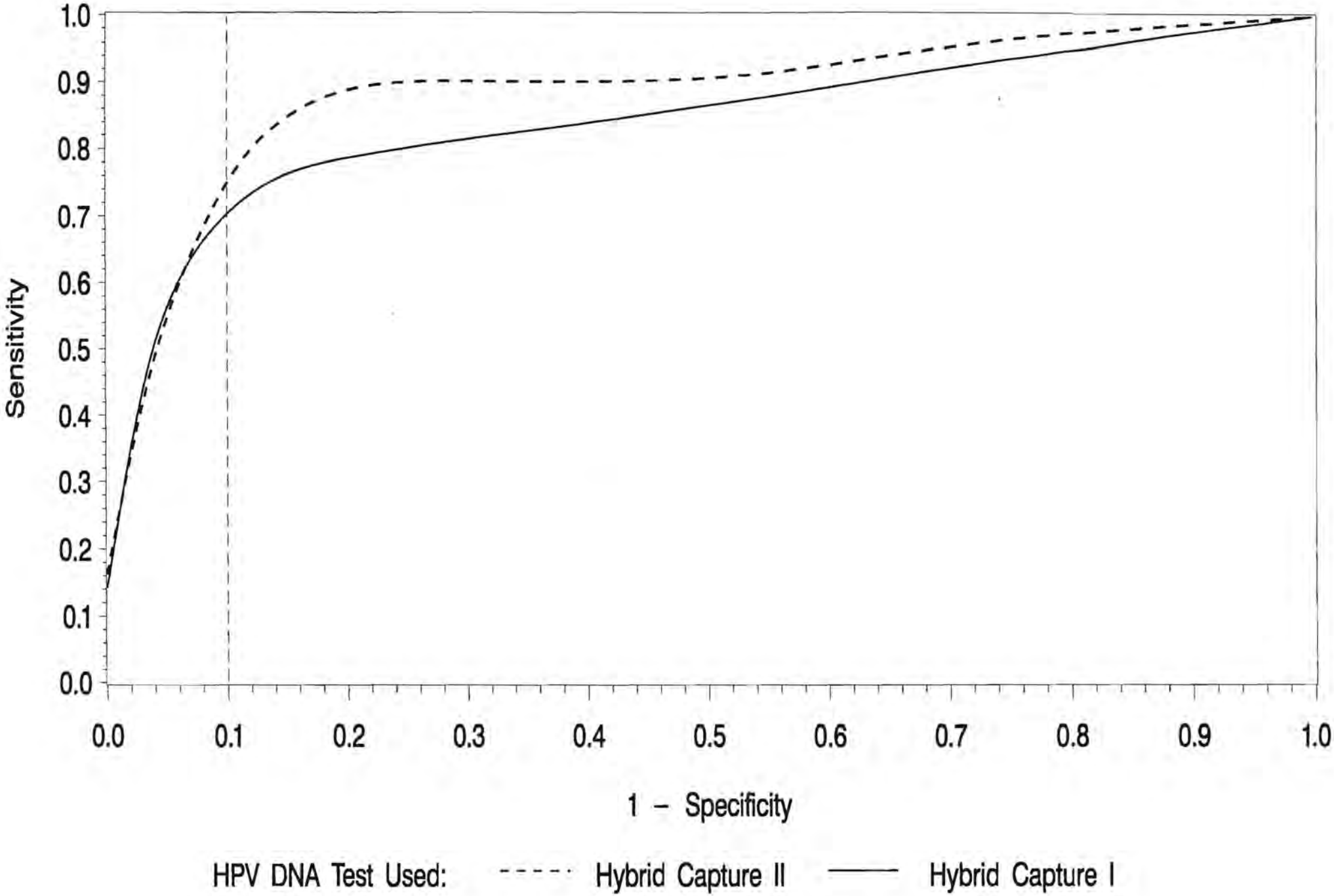
Table 27 shows the proportion of all cases of histologically confirmed HSIL and cancer that were HPV DNA positive using HC I and HC II using different cut-off values to define a positive HPV DNA test, such that the prevalence of HPV DNA in women without disease varies between 2.5 and 15%. Women with LSIL or with at least one positive screening test who did not receive colposcopy are excluded from the table.

HC II identified 88% of all cases of HSIL and cancer if the prevalence of HPV DNA detection in women without disease was allowed to increase to 15%, which occurred when 1x the positive control was used as the cut-off for a positive test. HC I reached a plateau of 73% detection of all cases of HSIL and cancer when the prevalence of HPV DNA in women without disease was 12%. At an HPV DNA prevalence of around 5% in women without disease, either test was able to identify over half (57% of the women with confirmed disease identified in this study).

| Type of HPV test | Proportion HPV positive among women without disease | Proportion HPV positive with HSIL or cancer | Cut-off value used to define HPV test as positive |
|---|---|---|---|
| HC I | 0.025 | 0.430 | 14.9 |
| | 0.050 | 0.570 | 4.8 |
| | 0.075 | 0.640 | 2.7 |
| | 0.100 | 0.709 | 1.4 |
| | 0.122 | 0.733 | 1.0 |
| | 0.125 | 0.733 | 0.9 |
| | 0.150 | 0.756 | 0.7 |
| HC II | 0.025 | 0.453 | 257.5 |
| | 0.050 | 0.57 | 119.2 |
| | 0.075 | 0.605 | 52.7 |
| | 0.100 | 0.791 | 8.3 |
| | 0.122 | 0.814 | 4.32 |
| | 0.125 | 0.837 | 2.34 |
| | 0.150 | 0.884 | 1.00 |

Table 27: Estimated proportions of all cases of HSIL and cancer classified as HPV positive and estimated HPV prevalence in women without disease (all screening tests negative or no disease after histological sampling) using different cut-off valued to define a test as positive


A
Ratio RLU sample : control
1000.00
100.00
10.00
1.00
0.10
No disease
Low grade SIL
High grade SIL
Cancer
5
Disease Category
Observed values
Geometric mean in category


Sensitivity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1 – Specificity
HPV DNA Test Used:
Hybrid Capture II
Hybrid Capture I

## 3.9 Follow-up Post-LEEP

### 3.9.1 Immediate Complications Post-LEEP

Bleeding at the time of LEEP was considered excessive (>100 mls) in 4.5% (n = 8), moderate (< 100 mls) in 49.4 % (n = 88) and no bleeding at all was documented 46.1% (n = 82) of the 178 women treated with LEEP. While 27.5% (n = 49) women complained of pain or cramping during the procedure, 5.1% (n = 9) of the women complained of dizziness or feeling faint. There were no serious complications.

### 3.9.2 Post-LEEP Follow-up

All women treated with LEEP were asked to return for follow up at 4- and 10-months post treatment. This group consisted of a total of 178 women: 149 women who had a LEEP at the first colposcopic examination, 28 women who had LEEP performed on the basis of a positive biopsy or ECC, one woman who had repeat colposcopy and LEEP on the basis of discrepancy between screening tests and the original colposcopy. In total, 141 (79.2 %) of these women returned for their follow up visit at 4 months and 64 (35.9%) returned for the second post-LEEP check up at 10 months.

### 3.9.3 Post-LEEP Follow-up at 4- and 10-months: Prevalence of SIL and Cervical Stenosis

Of the 141 women who returned for post-LEEP follow up at 4-months, 8 (5.7%) cases of cervical stenosis were diagnosed and no histological sampling was possible in these women. Overall, SIL was histologically diagnosed in 9 (6.4%) women who returned for their 4-month check up. Of the 64 women who returned for their 10-month post-LEEP follow up, there were a further 4 (6.3%) cases of cervical stenosis, one case of HSIL and 3 cases of LSIL

Tables 28 and 29 show the number of cases of SIL and cervical stenosis diagnosed at the 4- and 10-month check ups respectively in relation to the original LEEP diagnosis. Of the 51 women who had HSIL in the original LEEP specimen and who returned for the 4-month follow up visit, one woman had persistent HSIL (2.0%), 3 women had persistent LSIL (5.9%), 2 (3.9%) women had cervical stenosis and 45 women had no disease confirmed histologically. Of the 40 women who had LSIL in the original LEEP specimen and who returned for the 4-month check up, 3 had persistent LSIL (7.5%), there were no cases of HSIL and 3 (7.5%) women had cervical stenosis. Of the 49 women who had no SIL diagnosed in the original LEEP specimen, 2 had LSIL histologically confirmed at the 4-month follow-up (4.1%) and there were 3 (6.1%) cases of cervical stenosis.

| Histological Diagnosis in original LEEP specimen* | Histological diagnosis at 4-month follow up | | | |
|---|---|---|---|---|
| | No disease | LSIL | HSIL | Cervical Stenosis |
| No disease (n = 50) | 45 | 2 | 0 | 3 |
| LSIL (n = 40) | 34 | 3 | 0 | 3 |
| HSIL (n = 51) | 45 | 3 | 1 | 2 |
| Total (n = 141) | 124 | 8 | 1 | 8 |

Table 28: Histological diagnosis and cervical stenosis at 4 months post LEEP follow up [* includes only women who returned for post LEEP follow up]

Sixty-four women returned for the 10-month follow up and overall, 4 (6.3%) women had cervical stenosis, 3 (4.6%) had LSIL and one woman (1.2%) had HSIL. Of the 23 women who no disease in the original LEEP specimen, one woman (4.3%) had HSIL at the 10-month follow up and a further 3 (13.0%) women had developed cervical stenosis. Of the women with HSIL in the original LEEP specimen, 2 (8.7%) women had LSIL diagnosed at the 10-month follow up, no women had HSIL and there was one case of cervical stenosis (4.3%).

| Histological Diagnosis in original LEEP specimen* | Histological diagnosis at 10-month follow up | | | |
|---|---|---|---|---|
| | No disease | LSIL | HSIL | Cervical Stenosis |
| No disease (n = 23) | 19 | 0 | 1 | 3 |
| LSIL (n = 18) | 17 | 1 | 0 | 0 |
| HSIL (n = 23) | 20 | 2 | 0 | 1 |
| Total (n = 64) | 56 | 3 | 1 | 4 |

Table 29: Histological diagnosis and cervical stenosis at 10 months post-LEEP follow up [* includes only women who returned for post LEEP follow up]

### 3.9.4 Intermediate Complications of LEEP as Assessed at 4 month Follow-up Visit

Of the 141 women who came for post-LEEP assessment at 4 months, 24 (17%) reported that they had required medical treatment after the LEEP procedure. Of these women, 14 (58.3%) complained of pain and 7 (29.2%) complained of excessive bleeding and 3 women had unspecified complaints. Only one woman attended the emergency unit at Groote Schuur Hospital, and 23 women used primary care facilities.

## 3.10 Extrapolation of the Data into Different Screening Protocols

### 3.10.1 Introduction

One of the criteria that may enable successful screening in low resource settings, would be the elimination of colposcopic triage and histological sampling before treatment in the management of women with abnormal screening tests. Colposcopy and pathological services are generally not available in low resource settings and elimination of this step in the management of preinvasive disease may streamline the process, reduce the high default rate and enable on-site treatment. A particularly simple and attractive screening strategy would be to screen and treat women at the first visit, using a low technology screening test such as DVI.

This next section considers the possible outcomes of three different screening strategies, in which women would be treated, based on the screening test results, without the use of colposcopic triage.

Three possible approaches to screening include women being treated based on:

1 Positive screening tests used alone, in other words, a 'Test and Treat' protocol.
2 Selecting a subgroup of women who are positive on one screening test and then to perform a second screening test only if the first test is positive. If both tests were positive the women would be referred for treatment, so-called 'Two-Stage Screening'
3 Combining two screening tests, such that if either one or the other were positive, the women would be referred for treatment.

### 3.10.2 'Test and treat' Screening Protocol

Table 30 shows the percentage of women screened who would be correctly treated and over-treated by each screening test if women were treated on the basis of a positive screening test in the absence of colposcopy or histological sampling. HSIL and cancer would be correctly identified and treated in a similar proportion (approximately 2% of all women screened when any of the four screening tests were used). However, over-treatment of women without cervical disease would be more likely with a positive DVI and a positive HPV DNA test at the lower threshold for a positive test. For example, if women with positive DVI results were treated in the absence of colposcopy, 18% of the population would be treated and 14.4% of the screened population would be treated despite the fact that they are disease free. This represents 79% of the women with a positive DVI test (Table 29).

In contrast, if a positive cervical smear was used to determine who to treat in the absence of colposcopy, 8% of the screened population would be treated and 3.4 % of the total screened population would be treated despite being disease free, which represents less than half (42%) of the women with a positive cervical smear. HPV DNA testing at the 1x cut-off would perform similarly to DVI; 72% of

HPV DNA positive (1x positive control) women have no disease, but at the higher cut-off (10x) the test would perform similarly to cytology with 49% of women with a positive test having no disease.

| Screening test | Percentage of all women screened* | | | |
|---|---|---|---|---|
| | Total treated | Correctly treated | | Over-treated |
| | | HSIL or Cancer* | LSIL* | No disease+ |
| DVI | 18.1 | 2.1 | 1.7 | 14.4 |
| Cytology | 8.1 | 2.6 | 2.1 | 3.4 |
| CG™ | 10.5 | 2.0 | 1.7 | 6.8 |
| HPV DNA (10x) | 6.1 | 1.5 | 1.5 | 3.0 |
| HPV DNA (1x) | 16.2 | 2.3 | 2.2 | 11.7 |

Table 30: Percentage of all women screened who would be correctly treated and over-treated by each screening test in the absence of colposcopy (adjusted for lost to follow up) [* Percentage with LSIL, HSIL or cancer among those with a positive test result who underwent colposcopy multiplied by the percentage of women who were test positive on the screening test.
+Percentage with no histological evidence of SIL of any grade or cancer among those with a positive test result who underwent colposcopy multiplied by the proportion of women who test positive on the screening test]

Women called negative by the different screening tests would not be referred for treatment. From our data the number of known cases of SIL or cancer that would not be treated in a 'test and treat' screening protocol using DVI would be 1.2% of the women with a negative DVI test, 0.7 % of women with a negative cervical smear, 1.3 % of women with negative Cervigrams™, 1.8 % of women with a negative HPV DNA test (10x) and 1.0 % of women with a negative HPV DNA test at the 1x level. (These data are calculated from the overall disease prevalence, 3.3 % adjusted for lost to follow up minus those with disease who had positive screening tests).

### 3.10.3 Sequential or Two-Stage Screening

The six possible sequential pairs for 'Two-stage screening' include DVI and cytology, DVI and Cervicography™, DVI and HPV DNA testing, HPV DNA testing and Cervicography™, HPV DNA testing and cytology and Cervicography™ and cytology.

### 3.10.3.1 Sensitivities, Specificities, PPVs and NPVs for HSIL and Cancer of the Different Sequences in Two Stage Screening

The sensitivities and specificities of the different screening sequences for HSIL and cancer are shown in Table 31. Sequential testing would result in considerable loss of sensitivity compared to the four screening tests used alone. For the exception of HPV DNA detection and cytology, using the other

tests in sequence would have resulted in over half the cases of HSIL and cancer being missed. HPV DNA testing (1x) and cytology had a sensitivity of 67.5 (similar to DVI, cytology or HPV DNA testing (1x) when used alone) but had a far better specificity than DVI used alone (97 versus 83% respectively). Specificity was high for all six sequences. The order in which the tests were performed did not alter the results as calculated here. However, in a real situation, prior application of acetic acid would be expected to reduce the performance of cytology in women so screened.

While the sensitivities of the different sequences were markedly reduced compared to using the tests alone, the PPVs of the tests were significantly higher. A positive DVI and cervical smear had a PPV of nearly 50% compared to 11% when DVI was used alone. A positive DVI and positive Cervigram™ (both visual inspection methods) had the lowest PPV at 25% compared to 40% when Cervicography™ was combined sequentially with HPV DNA testing (1x) and 53% when Cervicography™ was combined sequentially with cytology.

| Screening tests – both positive | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| DVI+ Pap+ | 48.2 | 98.5 | 49.4 | 98.4 |
| DVI+ HPV+ | 44.2 | 96.9 | 30.9 | 98.2 |
| DVI+ CG+ | 48.1 | 98.2 | 25.0 | 98.3 |
| HPV+ Pap+ | 67.5 | 97.1 | 41.2 | 99.0 |
| HPV+ CG+ | 45.6 | 97.8 | 40.0 | 98.2 |
| Pap+ CG+ | 50.0 | 98.6 | 53.4 | 98.4 |

Table 31: Sensitivities, Specificities, PPVs and NPVs of the six different sequences for HSIL and cancer using the two-stage screening model [+ = positive; Pap = cytology; CG = Cervicography™, HPV + = HPV DNA testing (1x)]

### 3.10.3.2 The Percentage of Women Screened who would be Referred for Treatment and the Percentage of Women who would be Correctly Treated and Over-treated using the Six Different Screening Sequences in the Absence of Colposcopy

Table 32 shows the percentage of the women screened who would be referred for treatment and the percentage of women with positive screening tests who would be correctly treated or over-treated if the six screening sequences were used without colposcopy or histological sampling. The percentage of

women screened who would be referred for treatment is significantly lower for all the six sequences compared to using the screening tests alone. However, the percentage of cases of SIL or cancer correctly treated would also be decreased compared to using the screening tests alone, except for the sequence of HPV DNA testing and cytology.

In contrast to screening and treating women on the basis of positive screening tests alone, sequential screening reduces the number of women who are over-treated, particularly with the visual inspection methods of DVI and Cervicography™. For example, the sequence of a positive DVI test and a positive Pap will refer 3% of the population screened for treatment and 0.8% of all women screened would have no cervical disease and would be over-treated (representing 28 % of women with positive screening tests). By contrast, 79% of women with a positive DVI test used alone would be over-treated in the absence of colposcopy and histological sampling. A similar reduction in over-treatment is noted with the other five sequences.

| Screening sequences | Percentage of all women screened* | | | |
|---|---|---|---|---|
| | Total treated | Correctly treated | | Over-treated |
| | | HSIL or Cancer* | LSIL* | No disease+ |
| DVI+ Pap+ | 2.9 | 1.4 | 0.7 | 0.8 |
| DVI+ CG+ | 6.1 | 1.5 | 1.0 | 3.6 |
| DVI+ HPV+ | 4.2 | 1.3 | 0.8 | 2.1 |
| HPV+ Pap+ | 5.2 | 2.1 | 1.6 | 1.5 |
| HPV+ CG+ | 3.8 | 1.5 | 0.9 | 1.4 |
| Pap+ CG+ | 3.1 | 1.6 | 1.0 | 0.5 |

Table 32: Percentage of women screened using the six different sequences who would be correctly treated and who would be over-treated in the absence of colposcopy.(+ = positive; Pap = cytology; HPV = HPV DNA testing at the 1x level; CG = Cervicography™).

[*Percentage of women with LSIL, HSIL or cancer among those with a positive test result who underwent colposcopy multiplied by the percentage of women who were test positive on the screening test. +Percentage of women with no histological evidence of SIL of any grade or cancer among those with a positive test result who underwent colposcopy multiplied by the percentage of women who test positive on the screening test]

### 3.10.4 Combination of Screening Tests

Combining two screening tests such that if either one or the other test were positive women would be referred for treatment, is another possible screening strategy. The six combinations of tests include; a positive DVI or cervical smear, DVI or Cervicography™, DVI or HPV DNA (1x), HPV DNA (1x) or cervical smear, HPV DNA (1x) or Cervicography™ and a positive cervical smear or Cervicography™.

### 3.10.4.1 Sensitivities, Specificities, PPVs and NPVs for HSIL and Cancer of the Different Combinations of Screening Tests

The sensitivities, specificities, PPVs and NPVs of the different combinations of tests for HSIL and cancer are shown in Table 33. All the various combinations show significantly greater sensitivities compared to using the tests alone, but there is a corresponding decrease in specificity. The combination of a positive DVI or a positive cervical smear and a positive DVI or HPV DNA test (1x have equivalent sensitivity, although the specificity of the latter is lower.

A positive DVI or Cervicography™ test, both visual inspection methods of screening, has the lowest sensitivity and PPVs of the various combinations. Compared to sequential screening, the PPVs of the different combinations are much lower and are more or less equivalent to the PPVs of the screening tests used alone. The NPVs are nearly 100% for all the combinations.

| Screening combinations | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| DVI or Pap+ | 96.4 | 80.2 | 12.8 | 99.9 |
| DVI or CG + | 78.5 | 80.2 | 11.3 | 99.1 |
| DVI or HPV + | 96.5 | 72.8 | 9.9 | 99.9 |
| Pap or HPV + | 85.5 | 83.9 | 13.8 | 99.5 |
| Pap or CG + | 85.9 | 88.2 | 18.9 | 99.5 |
| HPV or CG+ | 87.3 | 80.5 | 12.6 | 99.5 |

Table 33: Sensitivities, specificities, PPVs and NPVs of the different screening combinations for HSIL and Cancer [+ = positive; HPV = HPV DNA testing (1x, Pap = cytology, CG = Cervicography™]

### 3.10.4.2 The Percentage of Women Screened who would be Referred for Treatment and the Percentage of Women who would be Correctly Treated and Over-treated using the Six Different Screening Combinations in the Absence of Colposcopy

Table 33 shows the percentage of the women screened who would be referred for treatment and the percentage of women with positive screening tests who would be correctly treated or over-treated if the six screening combinations were used without colposcopy. The percentage of women referred for treatment is significantly higher for all the six combinations compared to using the screening tests alone or sequentially. While the percentage of cases of SIL or cancer correctly treated would be increased compared to the other screening strategies, the percentage of women who would be over-treated is dramatically greater than using screening tests in sequence and similar to using the screening tests alone.

For example, while the combination of a positive DVI test or a positive cervical smear would refer 23% of women for treatment, 17.3% of the screened population would have no evidence of disease (representing 74% of women with positive screening tests, almost equivalent to treating women on the basis of a positive DVI test alone. Similar percentages of women over-treated without disease are noted in the other combinations.

| Screening combinations | Percentage of all women screened* | | | |
|---|---|---|---|---|
| | Total treated | Correctly treated HSIL or Cancer* | LSIL* | Over-treated No disease+ |
| DVI+ or Pap+ | 23.3 | 3.0 | 3.0 | 17.3 |
| DVI+ or CG+ | 23.0 | 2.6 | 2.5 | 17.9 |
| HPV+ or Pap+ | 18.0 | 2.5 | 2.6 | 12.9 |
| HPV+ or CG+ | 21.0 | 2.6 | 2.9 | 15.5 |
| Pap+ or CG+ | 14.0 | 2.6 | 2.6 | 8.7 |

Table 34: Percentage of women screened using the six different combinations who would be correctly treated and who would be over-treated in the absence of colposcopy (+ = positive; Pap = cytology; HPV = HPV DNA testing at the 1x level; CG = Cervicography™ )

[*Percentage with HSIL or cancer or LSIL those with a positive test result who underwent colposcopy multiplied by the percentage of women who were test positive on the screening test

+Percentage with no histological evidence of SIL or any grade or cancer among those with a positive test result who underwent colposcopy multiplied by the percentage of women who test positive on the screening test]

# Chapter 4
# Discussion

## 4.1 Introduction

This cross sectional study was performed on a volunteer sample of women living in a peri-urban settlement outside Cape Town. The main aim of the study was to define the comparative effectiveness of alternatives to cytological screening for the detection of cervical cancer and its precursors in a low-resource setting. Despite the relatively good performance characteristics of cervical cytology as a screening test for invasive and preinvasive disease, alternatives are being actively sought because it has proven difficult to introduce and maintain cytologically-based cervical cancer screening programmes in many resource-poor settings.

## 4.2 Study Sample

Since the study was designed to evaluate alternative methods for the detection of cervical cancer and its precursors in low-resource settings, it was important to locate the study among women of low socio-economic status living in a low-resource setting.

The characteristics of the study sample closely resembled the socio-economic and demographic profile of women living in Khayelitsha as described in the population-based survey by Cooper et al in 1991[1]. Of the women interviewed in this survey, 54% lived in serviced sites (compared to 67% of the women in this study). The same numbers of women were employed as in this study (31%) and in both studies the majority of the employed women were domestic workers. The number of women with no formal schooling was higher in this study (14% compared to 7% in the study by Cooper et al) but the number who had had some primary and some secondary level education were similar in the two studies. The similarity of the demographic profile of the women in the two studies suggests that our study sample was representative of the women of the Khayelitsha community and of women of low socio-economic status in general.

Selection bias in the study sample cannot be ruled out. While not formally evaluated, we conducted focus groups with women who had participated in the study in order to refine and develop educational material. During discussion in the focus groups, it became apparent that 'word of mouth' was a common source of referral to the study, in addition to our more formal recruitment strategy. One of the main reasons for this was that besides offering a screening service, the project personnel paid particular attention to the non-research related health needs of the women and provided a level of care not commonly encountered in the State-funded primary health care service.

For example, approximately 10% of the women who were enrolled in the study were referred to tertiary health care resources for further care, such as infertility, diabetic, hypertensive and general

gynaecology clinics. In the focus groups, women repeatedly expressed their satisfaction at having their 'other' health problems attended to. It is possible therefore that the study sample were relatively self-selected once knowledge became widespread that a fairly comprehensive health service was being offered by the project. This bias however, would not have affected the performance of the screening tests nor would it have prevented comparison of the performance of the screening tests in the context of a low-resource setting.

Women who had previously been screened were specifically excluded from the study in order to perform the study in a 'screen naive' population, such as would be encountered in many African countries. We restricted participation to women aged 35 to 65 years in order to capture the most high-risk women in the population. The fact that only 3% of the women recruited were over 60 years of age, may reflect the reluctance of this age-group of women to undergo gynaecological examination and a lower utilisation of health services in general.

The study population had many of the characteristics associated with populations at high risk for preinvasive and invasive cervical cancer. Over 50% of women had between 2 and 4 pregnancies, 66% had their first sexual encounter as teenagers and 67% had between 2 and 5 lifetime partners. Munoz et al[2] evaluated risk factors for CIN3 and carcinoma in situ in two case-controlled populations in Spain and Columbia In both Spain and Colombia, early age at first intercourse was associated with a significantly elevated risk of CIN 3. In addition, there was a significant increasing trend in risk of CIN 3 with increasing number of pregnancies, but this was only observed in Colombia. In both Spain and Colombia, the risk of CIN 3 increased with the number of lifetime sexual partners. The similarity in lifestyle characteristics of the women in our study and in those with established cervical disease in Spain and Colombia, indicates that our study sample were at particularly high risk for cervical disease.

Finally, the location of the project in an area within walking distance of most women's homes, meant that women without access to transport, those employed in the informal sector and unemployed women living in Khayelitsha had relatively easy access to the study site. Colposcopy was specifically performed on a Thursday afternoon as it is well known that many domestic workers have Thursday afternoons off work, making it easier for working women to return for colposcopic and follow-up examinations.

## 4.3 Strengths and Limitations of the Study

The strengths of this study were that the majority of the women (90%) with positive screening tests underwent colposcopy and histological sampling, all colposcopies were performed by the same colposcopist, which limits inter-observer variation, and all histology was reviewed, blinded to any clinical information, at a centre of excellence. Further, the screening tests were evaluated in the context of a population living in a low resource setting. Evaluation of screening test performance in other than the target population can produce considerable and unmeasurable bias, such as when screening tests are evaluated in a highly selected population as occurs in colposcopy clinics.

In addition, a trained nursing sister, rather than medical officers or gynaecologists performed all screening. Throughout Sub-Saharan Africa, the ratio of nurses to patients is consistently higher than the ratio of doctors to patients, particularly in rural areas. It is expected therefore that nursing sisters would be at the forefront of any newly established screening programmes in low-resource settings. The relatively high sensitivity of DVI in this study is an indication that nursing sisters can be trained to perform gynaecological examinations, to evaluate the cervix and to interpret their findings appropriately.

The use of community health workers (CHWs) who had lived in and understood the community to perform the enrolment, recruitment, education and tracking of women is another strength of the study. None of the CHWs had completed high school and two had previously worked as domestic workers, yet they were able to understand the nature and purpose of screening and to impart this knowledge to women in a manner that was accessible and understandable. Their closeness to the community engendered trust and possibly enhanced compliance with the requirements of the study. The on-site medical officer and the author constantly monitored the quality of the education provided by the CHWs to the women recruited to the study. The level of understanding shown by the women was considered remarkably good, however this was not formally evaluated. It did however, confirm the value of training relatively uneducated women to become CHWs and to perform fairly sophisticated tasks. This is important information and indicates that CHWs can and should be incorporated into the design of existing or any newly established cervical cancer screening programmes.

The main limitation of the study was that the 'gold standard' for detection of cervical disease, that is, colposcopy followed by histological diagnosis, was not applied to all study participants. The failure to apply the 'gold-standard' to subjects who test negative on screening tests, particularly if the procedure is invasive such as colposcopy and histological sampling, has been a recognised limitation of many screening studies. Some have even claimed that it is 'unethical to biopsy healthy women in order to establish the prevalence of disease in normal (control) populations'[3, 4].

The failure to perform colposcopy in women with four negative screening tests results in a form of verification bias and the true prevalence of disease in the screen negative women cannot be

determined[5, 6]. Verification bias refers to a distortion in the estimates of accuracy of the test when only selected subjects undergo disease verification with the 'gold standard' e.g. when only women with positive screening tests undergo colposcopy and histological sampling. In a meta-analysis of Pap test accuracy by Fahey et al[7] only 18% of reviewed studies verified disease status in all subjects. Pap test negatives were verified in a much lower proportion than those who were Pap positive and were often selected for disease verification on the basis of other positive test results, such as occurred in this study, or risk factors for cervical disease. In these circumstances, it is likely that verification bias will result in an over-estimation of sensitivity and an under-estimation of specificity[7].

In this study, the overall disease prevalence is low and we used four relatively independent screening tests to screen this population of women. We expect therefore, that the number of cases of disease missed in the screen negative women to be so low, that our estimates of sensitivity and specificity, while not entirely accurate, are a reasonable reflection of test performance.

In an attempt to quantify the degree of verification bias, we considered the sensitivity and specificity of DVI and HPV DNA testing using cytology as the 'gold standard'. We found the performance of the screening tests to be similar to that observed when judged against our combination of four negative screening tests or negative histology after colposcopic examination. Thus it appears that the extent of the verification bias was small.

A further limitation of this study, particularly in terms of generalising the results into clinical practice, is that the findings were made in the context of a closely monitored research study, with dedicated staff, ongoing training and audit. Whether the performance of the screening tests would be replicated in a day-to-day clinical setting, without the quality control built into a research study, remains to be evaluated.

In addition, the study provided a 'vertical' service to the women being screened, in that the study personnel were dedicated to recruitment, education, screening, treatment and tracking of women requiring follow-up. There is currently an intense debate in South Africa and other developing countries on the sustainability of 'vertical' health care programmes as opposed to 'horizontal' health care programmes. The latter programmes employ multi-purpose health workers operating from health centres and clinics, rather than personnel dedicated to specific health problems. The high level of compliance achieved in this study is a reflection of what can be achieved with dedicated staff. Whether this could be sustained in a poorly-resourced health care infrastructure is untested, another factor that needs careful consideration in the establishment of screening programmes.

## 4.4 Association of Socio-demographic Characteristics with HSIL and Cancer

Epidemiological studies have identified a number of risk factors for the development of both cervical cancer and its precursors. These include early age of first intercourse, age of first pregnancy, number of sexual partners, cigarette smoking, oral contraceptive use, socio-economic class, interval since the last Pap smear, a history of abnormal Pap smear, high parity, immunosuppression and infection with Herpes simplex virus Type 2 or specific types of HPV (i.e. types 16 and 18)[8-17].

Although the risk factors for cervical cancer and its precursors are similar, the strength of the association between these risk factors and cervical cancer is generally stronger than between the risk factors and SIL. The two major independent risk factors identified for cervical cancer precursors have been lifetime number of sexual partners and cigarette smoking[18, 19]. Some studies have also identified early onset of sexual activity to be an important risk factor[20]. Harris et al[21], in an attempt to disentangle correlated risk factors for preinvasive lesions, found that the number of sexual partners as a risk factor exerted effects independently of age at first intercourse, whereas the reverse was not true. They concluded that the role of adolescent sexual activity as a risk factor for preinvasive lesions was unsupported by their data.

In this study, the only risk factor that achieved statistical significance after multivariate logistic regression analysis was the association between ever use of long acting injectable progestogen contraception use (LAIP) and HSIL (OR 2.17, 95%CI: 1.29 – 3.64). This association was stronger when ever use of LAIP was analysed specifically controlling for age, smoking and being currently sexually active (OR = 2.08; 95%CI: 1.21 – 3.58).

Published data on the relationship between LAIP use and cervical cancer and its precursors is inconclusive. Herrero et al[22] in a case-controlled study, found that the RR of invasive cancer in women who had used injectable contraceptives for 5 years or longer was 2.4 (95%CI: 1.0 – 5.7). The effect of prolonged use of LAIPs was stronger for women reporting use for 10 or more years (adjusted RR 3.4, 95%CI: 1.1 – 24.9) and among women who reported never having had a Pap smear or having had a Pap 2 or more years before the interview (adjusted RR 6.3, 95%CI: 2.1-18.7). However, women reporting use of LAIP for less than 5 years had an adjusted RR of 0.5 (95%CI: 0.3 – 0.9). The reduced cervical cancer risk associated with short-term use of LAIPs may have related to more intensive screening when this method of contraception was initiated.

In contrast, a WHO Collaborative Study of Neoplasia and Steroid Contraceptives, which conducted a large hospital-based case-control study in Thailand, Mexico and Kenya, found no increased risk in cervical cancer in women who ever used LAIPs, nor did they find any increased risk in duration of use of LAIPs[23].

However, in the same WHO study, the relationship of LAIP use to the risk of carcinoma-in-situ showed an elevated risk in women who had ever used LAIP and the risk increased with duration of use of LAIPs[24]. In addition, decreasing trends in relative risk with times since first and last use of LAIPs were observed in long-term users. Their findings suggested that if LAIP increase the risk of carcinoma-in-situ, then either this was a reversible effect, or the cervical lesions induced by LAIP tended not to progress to invasive disease.

Similar to the WHO collaborative study, a case-control study in Costa Rica in 1988 showed no increased risk of invasive cancer or of carcinoma-in-situ in women who used LAIPs. However, very few women in the study had used ICs for longer than 2 years[25].

The association between LAIP and HSIL noted in this study may be explained by the relatively strong relationship between HPV infection of the cervix and use of LAIP. In our study, this relationship was statistically significant on univariate but not multivariate analysis controlling for age, education, housing type, marital status, current sexual activity, lifetime number of partners, age of first sexual intercourse, parity and cigarette smoking. The relationship however between current sexual activity and HPV infection of the cervix was significant after controlling for the same variables (OR 1.4: 95%CI: 1.07 – 1.84). It is possible that the increased risk of HSIL in ever uses of LAIPs observed in this study, represents greater sexual activity among contraceptive users, rather than a direct effect of LAIP on the pathogenesis of HSIL. This hypothesis would need to be tested on a much larger sample of women to be validated.

Of the women who failed to return for colposcopy, the majority were women who lived in unserviced sites, women who used alcohol and nulliparous women. Women living in unserviced sites represent the poorest and most marginalised women in the community, a group for whom health care, particularly preventative health care, is unlikely to be a priority. Use of alcohol among women in the Khayelitsha community is uncommon and women who do use alcohol, most likely represent women who are significantly socially dislocated. It is likely that both groups of women are at high-risk for cervical disease. A number of studies have suggested that women at highest risk for cervical disease are least likely to attend screening programmes [26 - 28]. Efforts to improve compliance among this group of women need to be developed for each health care setting. Providing on-site screening and treatment for these women enabled these women to come for screening, but did not overcome their resistance to compliance with the screening programme.

## 4.4 Summary of Main Findings with respect to Screening Test Performance

This study has found that none of the non-cytological methods of cervical screening, when used alone provide the same combination of high sensitivity and specificity as the cervical smear. However, the high sensitivity and specificity of cytology in this study may not be reproducible outside the research context. A recent comprehensive meta-analysis of all published cervical screening studies from January 1984 to March 1992, which consisted of 62 studies, estimated the sensitivity of cytology in screening studies to be between 49 - 67%, with specificities ranging from 62 – 77%. Further, from ROC analyses, sensitivities as low as 20 – 35% were recorded if specificities of 90 – 95% were achieved[7].

In this study, cytology performed better than reported in this meta-analysis with a sensitivity of 78% and a specificity of 95% for HSIL and cancer. However, the cervical smears were taken by a well-trained nursing sister under ideal conditions i.e. women were examined in the lithotomy position with good quality lighting and quality sampling devices. In addition, the smears were read by highly trained cytotechnologists, who were aware that the smears were part of a research study, and the smears were read in a laboratory attached to an academic institution with stringent built-in quality control.

Although both DVI and HPV DNA testing (at 1x positive control) had an acceptable level of estimated sensitivity for HSIL and cancer (67 and 73% respectively) and statistically equivalent to that of cytology at 78%, both tests had a considerably lower specificity than cytology at 84 and 89% respectively. The relatively low specificity of these two tests would have resulted in many women without cervical disease being classified as disease positive. This would overburden the colposcopic services if colposcopy were used to triage women with abnormal screening tests or would result in considerable over-treatment of women lacking cervical disease if a 'Test and Treat' policy were followed. Although HPV DNA testing (at 10x the positive control) had an estimated specificity equivalent to that of cytology (both 95%), the test would identify only half the cases of HSIL or cancer in the population screened. HPV DNA testing using HC II had the highest sensitivity for HSIL and cancer of all the screening tests, but with the expected 'trade-off' of a lower specificity. Cervicography™ performed only marginally better than HPV DNA testing (at 10x positive control) with sensitivity of 58% for HSIL and cancer, and a lower specificity than cytology at 92%.

In contrast to sensitivity and specificity, which cannot be estimated with certainty unless the 'gold standard' is applied to all women screened, the positive predictive value (PPV) of the tests is not influenced by this limitation. The PPV of a test indicates the likelihood of having disease given a positive test.

While DVI and HPV DNA testing (at 1x the positive control) identified the highest number of women as having a positive test (18 and 16% respectively), both tests had a low PPV for biopsy-confirmed HSIL and cancer at 11 and 14% respectively. In contrast, using HPV DNA testing but only classifying women with high viral loads (> 10 x positive control) as having a positive test, far fewer women were

identified as having a positive test at 6% and nearly double the number, 25%, of women had biopsy confirmed HSIL or cancer. Cervicography™ on the other hand performed better than DVI, but only marginally by identifying 11% of women as having a positive test, of whom 19% had HSIL or cancer. In contrast, cytology identified 8% of all women screened as having a positive test and HSIL and cancer were histologically confirmed in 32% of the cases.

The NPV, which is a measure of the likelihood of having no disease given a negative screening test, was uniformly high for all the screening tests. A high NPV is an important characteristic of a screening test as it suggests that women with negative screening tests are very unlikely to harbour undetected disease.

The performance of the screening tests in terms of sensitivity, PPV and NPV was not influenced by age. The specificity however of both visual inspection methods, that is DVI and Cervicography™ increased significantly in the 50 – 65 year age group. This is most likely due to the absence of active metaplasia in older women, leading to fewer false positive calls. Similar findings with regard to the specificity of Cervicography™, were reported in a study of 8460 Costa Rican women who were screened with cytology, HPV DNA testing and Cervicography™ [26]. Their data showed a significant decrease in sensitivity of Cervicography™ with age, but an increase in specificity. The decrease in sensitivity was most likely due to lesions being 'hidden' in the endocervical canal and was strongly related to menopausal status. In our study however, sensitivity of Cervicography™ did not decrease appreciably with age.

In summary, DVI, HPV DNA testing (at 1x positive control) and cytology had similar sensitivities for HSIL or cancer, but the specificity of cytology was much higher than either DVI or HPV DNA testing (at the1x cut-off). In terms of sensitivity, Cervicography™ and HPV DNA testing (at 10x positive control) performed the worst, although both tests had higher specificities than either DVI or HPV DNA testing (at 1x the positive control). No test had a PPV above 50% for HSIL or cancer, although cytology had a PPV of just under 60% if all cases of SIL (including LSIL) were included in the calculation. HPV DNA testing using HC II had the highest sensitivity of all the screening tests.

## 4.5 Direct Visual Inspection of the Cervix after Application of 5% Acetic Acid

Visual screening methods as alternatives to cytology in low-resource settings have received considerable attention, as they are perceived to require a lower level of infrastructure and to be less expensive than cytology. They do not require laboratories with their attendant training and quality control costs. In addition, they provide an immediate result, an advantage in settings where women live considerable distances from health clinics or where it is difficult to track women who have abnormal test results.

Many studies of DVI that have incorporated the use of acetic acid washing of the cervix have consistently found the sensitivity of DVI to be equivalent to that of cytology for detecting HSIL or cancer. These studies have enrolled women from diverse clinical settings including Italy, India, Zimbabwe, South Africa and the United States. The results obtained with DVI have been remarkably consistent given the diverse clinical settings[27 - 31].

This study confirms these results and extends them by demonstrating that the use of low magnification (2.5x) does not enhance the sensitivity of visual inspection. Not a single case of disease was identified using magnification that was not also identified by the nursing sister using her 'naked-eye' alone.

While the sensitivity of DVI for HSIL and cancer is acceptable at 67%, the specificity and PPV of the test are both low. These findings have important implications for a screening programme, particularly if DVI were to be used in a 'Test and Treat' protocol, because large numbers of women would undergo treatment to prevent cervical cancer in a small proportion of women in the screened population. The impact of such a protocol cannot be assessed until a comprehensive cost-benefit analysis is performed and the consequences of over-treating large numbers of women are clearly defined in terms of side effects, outcome of treatment and acceptability to the target population.

One important question is whether the specificity of DVI can be improved?

### 4.5.1 Methods of Increasing the Specificity of DVI

**1] Development of standardised training and interpretation of aceto-white lesions**

Possible methods of increasing the specificity of DVI include the development of standardised and reproducible criteria to define a positive test, such as is done with colposcopy, namely the Reid Colposcopic Index[32]. In our study the nursing sister was trained to identify all aceto-white lesions without attempting to grade the severity of the lesions. In India, Sankaranarayanan et al[29] trained paramedical staff to differentiate significant from insignificant aceto-white lesions. The screeners, who were cytotechnicians, were trained to grade the aceto-white lesions in the following way: the test was considered positive only if a distinct aceto-white area was detected. If the aceto-whitening was doubtful or faint, the test was scored as negative. DVI was considered positive in just under 10% of the women screened compared to 18% in the Khayelitsha study where the nurse was not trained to differentiate 'mild' aceto-white from more distinct aceto-white lesions. In addition, in the Indian study, DVI detected 90.1% of the true positive lesions, with an estimated specificity of over 90% compared to 83% in this study. In the Zimbabwe cervical screening project, screeners were also not taught to grade the severity of the aceto-white lesions[31]. Twenty per cent of the women screened were called DVI positive with a sensitivity of just over 70% (similar to this study) but with an even lower specificity of 64%.

Sankaranarayanan et al[29, 30] suggest that improved techniques for performing DVI of the cervix could reduce the false positive rate of DVI and improve specificity without compromising sensitivity. For instance, they recommend training staff to recognise artefacts due to glare from the light source, to wipe away mucus and cervical secretions and only to refer those women with dull aceto-white areas that cannot be wiped away. Larger studies are needed to prove that this approach is reproducible and sustainable.

**2] Pre-treatment of cervico-vaginitis**

This study found that visible inflammation of the cervix was associated with a significantly higher sensitivity for HSIL and cancer but there was a concomitant decrease in specificity of DVI. Lower genital infections, which cause cervical inflammation, may be the cause of the high false positive rate of DVI or low specificity. In our study, over 80% of women were noted to have a significant vaginal discharge and in just under half of these women the discharge was characterised as severe.

In phase two of this study, which is an extension of the study presented in this thesis and in which a similar population of women were screened, women were tested for T. vaginalis infection, as well as endocervical infection with either C. trachomatis or N. gonorrhoea [Hybrid Capture GC/CT DNA Assay, Digene Diagnostics, Silver Spring, MD]. Of the women screened, 20% had culture proven Trichomonas infection and 8% had C. trachomatis or N. gonorrhoea infection of the cervix, indicating a high prevalence of cervico-vaginal infection in this population of women[35].

Whether it is possible to decrease the false positive rate of DVI by pre-treating women with clinical cervicitis with antibiotics is unclear and there are no published data to support this approach. The only available data on this approach is preliminary data from an ongoing study in Kenya, based in a family planning clinic. DVI was falsely positive in 17/30 women pre-treated with antibiotics compared to 23/35 women who were not pre-treated with antibiotics ($p = 0.5$) suggesting that pre-treatment with antibiotics did not influence the outcome of DVI [Personal communication, Dr Hugo De Vuyst, Kenyan Screening Study]. Clearly pre-treating all women before screening would greatly increase the costs and complexity of the screening process.

**3] Use of DVI in Sequential or Two Stage Screening**

The Two-Stage Screening approach incorporating two sequential screening tests (the second performed only if the first is positive), followed by immediate treatment of women positive on the two tests performed in sequence, could reduce many of the obstacles to cervical cancer screening experienced in low resource settings and improve the specificity of DVI testing.

For example, using data from the Khayelitsha study, DVI followed by either an HPV DNA test or a Pap smear, would dramatically reduce the number of women referred for treatment, from 18% to less

than 5 % of the screened population. In addition, specificity is greatly enhanced, from 84% if DVI is used alone to over 97 – 99 % for DVI followed by either HPV DNA testing cytology or respectively. The 'pay-off' for the increased specificity is a reduction in sensitivity from 67% to 48% for the detection of HSIL and cancer. In a true low-resource setting, where facilities for screening do not exist, this low sensitivity may be considered acceptable. The high specificity means that far fewer women without disease will be unnecessarily treated, while just under half of the disease in the screened population would be detected.

Although Two-Stage Screening will dramatically reduce the number of more sophisticated screening tests required by the screened population, the infrastructure to perform the more sophisticated screening tests will still be required. With Two-Stage Screening this infrastructure could be centralised to save costs, but will introduce the same problems associated with delayed results and recall of women with abnormal tests. However, since only women with a positive DVI test will be selected for a second test, resources could be focussed on these women for further testing and surveillance rather than the entire screened population.

### 4.5.2 Service Implications of DVI

While DVI is a low technology screening test, it requires trained and committed personnel. Gynaecological examination of women with a speculum and performing DVI require a relatively high degree of commitment and training of nursing staff. In busy primary health care clinics, which typically in low-resource settings are under-staffed, it may be difficult to motivate staff to perform this apparently simple procedure.

This also raises the question as to whether screening can be performed in an integrated fashion, at primary care level or whether a DVI-based screening service would need dedicated staff and facilities. The Khayelitsha study was delivered as a classical 'vertical' service, with all staff dedicated to recruitment, screening and tracking of women with abnormal results when required. In the context of a busy clinic with limited staff it may not be possible to provide such a service. In future studies, we will be evaluating the service delivery implications of DVI, specifically looking at the advantages and disadvantages of integrating DVI screening into primary care compared to establishing a vertical screening process, with separate and dedicated staff.

### 4.5.3 Quality Control of DVI

Another important limitation of DVI is the lack of standardised and reliable quality control. In this study, quality control of DVI was through assessment of the appropriateness of referrals at colposcopy. In a setting where DVI would be used without colposcopy, the only form of quality control would be reduction in the incidence of cervical cancer in the screened population, data that would take years to collect and in the absence of a national cancer registry, would not be available at all. Ongoing training

and evaluation of trainers would introduce a further method of quality control and this in itself will require additional resources.

### 4.5.4 Repeated DVI Testing

One of the debates about screening revolves around screening intervals. The false negative rate of cytology has been shown to decrease if the test is repeated at regular intervals[34 - 38]. In a study conducted by WHO and IARC in 1985 and reported by Eddy[38], it was estimated using mathematical modelling, that screening 100% of the female population aged 35 – 64 years every year with cervical cytology, would reduce the cumulative incidence of cervical cancer by 93%. Screening women every 3 years would reduce the incidence by 91%, every 5 years by 85% and every 10 years by 64%. The Khayelitsha study evaluated DVI as a once off screening test and we cannot comment on the cumulative impact of repeated DVI testing. For countries such as South Africa, screening women every 10 years, that is, providing all women aged 30 – 60 years with 3 free cervical smears in a lifetime has been estimated to be affordable[39].

DVI, being an apparently far cheaper and more implementable test than cytology, may enable more frequent testing than cytology, even yearly if resources permitted. We can only speculate on the impact of yearly or three yearly DVI testing on the incidence of cervical cancer, but if the false negative rate of the screening process decreases with repeated testing and the high sensitivity found in many DVI studies is reproducible, the impact may be considerable. Only a detailed cost-benefit study and implementation of DVI in the field would enable this question to be answered.

In addition, DVI was not evaluated as a screening test post treatment for preinvasive lesions and there is no published data on the utility of DVI in this context. In a screening programme based solely on DVI for both primary screening and post-treatment follow up, this aspect will need to be carefully evaluated.

### 4.5.5 Conclusions: DVI as a Primary Screening Test

This study has shown that DVI performs equivalently to cytology in terms of sensitivity and will detect the majority of high-grade preinvasive and invasive lesions in the screened population. We have also shown that a trained nursing sister in a primary health care clinic setting can perform the test adequately. There remain however, many unanswered questions about the utility of DVI as a screening test for a national population screening programme, particularly if DVI were to be performed in the context of 'Test and Treat' protocol, without the use of colposcopy and histological sampling. For instance, what are the implications of over-treating large numbers of women? Would it be acceptable, in order to prevent cervical cancer, that nearly 80% of all women undergoing treatment do not have disease? Would women find this acceptable? Would the incidence of cervical cancer be reduced by this

strategy? What is the cost-benefit ratio? Can DVI screening be introduced into an integrated primary health care setting or does it require a vertical structure?

The answers to these questions may lie in the level of health care infrastructure available to different health care settings. However, a screening test that subjected large numbers of women to a treatment intervention that had significant side effects and complications, and was unacceptable to the women being screened, would not be acceptable under any circumstances. In the future, we will be evaluating the consequences of using DVI in a 'Test and Treat' protocol, in a randomised prospective study, in which nursing sisters will be trained to perform cryosurgery on women with positive DVI tests. The main outcome measures will be reduction in the incidence of HSIL, complications and acceptability of the screening and treating process. These data will hopefully answer the questions posed here.

## 4.6 HPV DNA testing as a Primary Screening Test

### 4.6.1 Introduction

Despite the evidence that specific types of high-risk HPV are causally associated with the development of cervical cancer, relatively limited data are available on the use of HPV DNA testing as an alternative screening method. There are a number of reasons why HPV DNA testing has not been widely adopted for cervical cancer screening. Foremost among these is the natural history of HPV infections. Anogenital HPV infections are common in young, sexually active populations[40, 41]. For instance, Ho et al[40] followed 608 college women (mean age 20 years) and found that the cumulative incidence of HPV DNA infection, as measured by PCR and included testing for all known high-risk types of HPV, was 43% (95% CI: 36 – 49). The median duration of infections however, was only 8 months (95% CI: 7 – 10).

Further, infection, even with high-risk types of HPV, such as HPV 16 or 18, does not always lead to the development of SIL. The majority of HPV infections in young women are transient and only a minority of HPV-infected women develop persistent infections[42]. Even women who are persistently infected with HPV DNA can manifest variable shedding of the virus over time. Hildesheim et al[43], followed a population of 393 middle class women with normal Pap smears, mean age 26 years, and tested for multiple HPV DNA types using PCR. Of those women infected with HPV 16, only 50% had persistent infection over a 14-month period.

Nobbenhuis et al[44] showed in their study of 353 women with abnormal Pap smears (mean age 32 years, range 18 – 55), that the overall median clearance time of high-risk HPV was 25 months. After 5 years of follow up, 67% of women had cleared their infections. Further the median time of clearance of newly acquired HPV was 6 months. This relatively long overall clearance time may be due to the fact that large numbers of women had HSIL, a group of women who seldom clear HPV infection.

Another reason why HPV DNA testing has not been adopted for routine cervical cancer screening is that the earlier commercially available HPV DNA molecular diagnostic tests lacked the required sensitivity. Recently, robust, highly sensitive HPV DNA tests have become commercially available such as Hybrid Capture I and II. These tests have sensitivities that have been shown equivalent or superior to that of cytology in detecting high-grade SIL and cervical cancer[45, 46, 47]. The availability of these highly sensitive tests offers the potential for replacing conventional cytological screening in settings where cytological screening programmes have not been successful.

### 4.6.2 Advantages of HPV DNA Testing

HPV DNA testing offers a number of theoretical advantages over cytological screening in selected settings. In contrast to cervical cytology, which is highly subjective, HPV DNA tests are standardised,

objective and provide a quantitative determination of the amount of HPV DNA present in a sample. A mid-level technician can test large numbers of samples for HPV DNA per day, whereas cytological screening requires highly trained cytotechnicians who can evaluate only 55 – 80 specimens per day[48]. In addition, cervical inflammation and cervico-vaginitis are not known adversely affect the results of an HPV DNA test, which are common in women in low-resource settings. However the ultimate clinical acceptability of a screening test depends on the sensitivity and specificity of the test in the target population.

### 4.6.3 Sensitivity and Specificity of HPV DNA Testing

The sensitivity of HPV DNA testing (at 1x the positive control) was equivalent to or better than cytology in this study, eventhough cytology performed exceptionally well in this study and better than reported in the meta-analysis by Fahey et al[7]. The estimated specificity of HPV DNA testing at the standard cut-off value for a positive test (1x) was however, lower than that of cytology in this study. Comparable specificity to cytology could only be achieved if the sensitivity of HPV DNA testing was allowed to decrease to around 57% by using more stringent, higher cut-off values for a positive test.

Of the two HPV DNA assays evaluated in this study, the second generation HC II assay was superior, in some respects, to the first generation HC 1 assay. High-risk HPV DNA was detected in 88% of women with HSIL and 100% of the women with cancer using the HC II assay, compared to 72% of the cases of HSIL and 83% of the cancers using the HC I assay. When the cut-off value of HC II was shifted to a point at which its sensitivity was equivalent to that of HC 1, the specificity of HC II was marginally better than that of HC I. In addition, the better performance of HC II compared to HC I is reflected in the stronger associations between HPV prevalence and cervical disease observed using HC II compared to HC I. However, although HC I was not able to achieve the same degree of sensitivity as HC II, at specificities likely to be practical for a primary screening test in a low-resource setting, HC I performed equivalently to HC II.

In the context of screening, good sensitivity (i.e. the ability of the test to detect all women with the condition of interest) has to be balanced against the specificity of the test. Specificity is particularly important in cervical cancer screening because screening, by its nature, requires large numbers of otherwise healthy women to be tested, and positive screening tests result in a follow up colposcopic examination that is both uncomfortable for women and costly. Specificity takes on added importance in low-resource settings where colposcopy is not available and where all women who are classified as positive by a screening test may undergo treatment. One of the advantages of HPV DNA testing in these settings is that the specificity of the test can be altered by adjusting the cut-off level used to define a positive result. For example, using ROC analysis, if over-treatment of 10% of women with no evidence of cervical disease were acceptable, then HC II could detect 79% of the cases of HSIL and cancer. If over-treatment of only 5% of women with no evidence of disease were acceptable, then both tests, HC I or HC II, could identify 57% of the cases of HSIL or cancer.

### 4.6.4 Other Studies of HPV DNA Testing as a Primary Screening Test

There have been very few studies on the use of HPV DNA testing as a primary screening test. Cuzick et al[49] performed HPV DNA testing using a semi-quantitative PCR assay and cytological testing on 1985 women who presented for routine cervical screening between 1992 and 1994, median age 29 years. HPV testing was for types 16, 18, 31 and 33. HPV DNA testing had a sensitivity for HSIL of 75% and a PPV of 42% compared to a sensitivity of 56% for cytology (any grade of SIL used for colposcopic referral), with a PPV of 35%.

In a later study, 2988 women, aged 34 years and older, underwent cytology and HPV DNA testing for 10 high-risk types of HPV, using the consensus PCR/SHARP assay[50]. The sensitivity of cytology for HSIL in this older population of women was 62%, with a PPV of 63%. Overall, 6% of the samples were positive for high-risk HPV using the PCR/SHARP assay. The sensitivity of HPV DNA testing for high-grade disease was 73.8%, with a PPV of 17.4%. In addition to testing with PCR/SHARP assay, women who were positive on the PCR assay or cytologically positive and a sample of controls negative on both tests were retested using HC I and the newer generation HC II test. The HC I test had a slightly better sensitivity than the PCR assay, but a very poor specificity leading to an 18% false positive rate. However, the HC II test, which was used in 60% of the study population, had a sensitivity of greater than 95% and a false positive rate of 4.9% when used at the recommended cut-off level of 1 pg/ml. All the HPV DNA positive high-grade lesions had a value in excess of 4 pg/ml, and if a 2 pg/ml cut-off were used, these was no loss of sensitivity and the false positive rate was reduced to 2.3%.

Schiffman et al[51] reported on a much larger study of the screening performance of HPV DNA testing as a primary screening test on a cohort of 8554 Costa Rican women. Using the HC II assay and the low cut-off for a positive test (1x positive control), 88.4% of the 138 high-grade lesions and cancers were detected using HPV DNA testing (all 12 cancers were HPV positive), with a colposcopic referral rate of 12.3%. Specificity was 89%. By comparison, Pap smear testing, using ASCUS as a cut-off point for referral resulted in a sensitivity of 77.7% and a specificity of 94.2% with a referral rate for colposcopy of 6.9%. In addition, by analysing ROC curves by age tertiles, HPV testing was shown to be optimal at older ages, where sensitivity was maintained with increased specificity and reduced rates of referral for colposcopy (21, 11.2 and 7.1% in each advancing tertile of age respectively).

The finding that the performance of HPV DNA testing in a high-risk, previously unscreened population is equivalent to that of expert cytology has important implications for countries that have not yet developed national cervical cancer screening programmes. In many settings, it may prove easier to establish clinical laboratories for large-scale HPV DNA testing than to establish high quality cytology laboratories. HPV DNA testing requires less skilled technicians and is easier to perform than cervical cytology. In addition, HPV DNA testing not only identifies women who currently have high-grade cervical disease, but also identifies those women who are at greatest risk of developing disease in the

future. HPV DNA testing would thus allow follow up efforts to be targeted to women at greatest risk for developing cervical disease in the future. Cost effective analysis of HPV DNA testing is urgently needed in order to evaluate its impact on cervical cancer prevention in various health care settings.

### 4.6.5 Two-Stage Screening

Similar to DVI testing, the specificity of HPV DNA testing is significantly enhanced by Two-Stage or sequential screening, for all possible sequences involving HPV testing. HPV DNA testing followed by cytology only for those with a positive test (at 1x the positive control) had a sensitivity for HSIL and cancer almost equivalent to cytology at 68% with a higher specificity (97%) than when cytology was used alone. In addition, the PPV was also higher at 41%, with a NPV of 99%. Further, based on our data, this sequence of tests would refer only 5% of the women screened, compared to 16% if HPV DNA testing were used alone.

The sequence of a positive HPV DNA test followed by a Pap smear, would require cytological services for only 16%, as opposed to 100% of the population were cytology to be used as the only screening test. If this quality of test performance were to be replicated in a day-to-day clinical setting, it would offer a very high quality screening strategy. In order though, to institute national population screening with HPV, the logistics of implementing widespread HPV testing would have to be evaluated on a larger scale. If however, with the rapid development of modern molecular technology, a 'dipstix' type of HPV DNA test could be invented, and assuming it was affordable, this method of screening could revolutionise screening in poor countries. Very low resource countries may still not be able to establish quality cytology, and in those settings, Two-Stage Screening using DVI may be an option.

HPV DNA testing in sequence with DVI testing, also achieves a very high specificity and an improved PPV, but at the cost of a significantly lower sensitivity. However, the very high NPV of 98%, would enable women with negative HPV DNA tests, followed by a negative DVI to be safely excluded from the screening programme, probably for as long as 10 years.

### 4.6.6 Prevalence of HPV by Age

The prevalence of high-risk HPV DNA was very high in this population of older African women. High-risk HPV DNA was detected in 16% of women with HC I assay and 22% of women with the HC II assay using the standard cut-off (RLU >1x positive control) to define a positive test, and relatively high levels of high-risk HPV DNA were present in many of these women. Furthermore, the prevalence of HPV DNA did not decrease significantly with age, although the prevalence followed a u-shaped curve with the lowest prevalence found in the 40 – 49 year age group. In women with no evidence of disease (four normal screening tests or no disease after colposcopy and histological sampling), 12 %

(using HC 1) and 18% (using HC II) were HPV DNA positive. These prevalence rates are much higher than those reported from studies on women in developed countries.

Melkert et al[52] investigated the prevalence of HPV DNA in four groups of women with cytologically normal smears. The first group consisted of young women aged 15 – 34 years visiting their doctors for check-ups, the second, were women aged 35 – 55 years participating in a triennial screening programme. The third and fourth groups were women attending a gynaecological outpatients department and were aged 15 – 34 and 35 – 55 years, respectively. The prevalence of high-risk types of HPV was 3.8 and 3.3% in the younger age groups and 0.9% and 1.5% in the two older age groups respectively ($p< 0.001$). The highest prevalence of HPV types 16 and 18 was in the 20 – 24 year age group at 8 – 10 %. In the older women, the prevalence of HPV DNA was much lower. However, similar to the prevalence of HPV DNA across the age groups in our study, HPV DNA prevalence followed a u-shaped curve in women between 35 and 55 years.

Meijer et al[53] reported on HPV DNA testing by PCR of women engaged in a population-based screening programme, aged 35 – 55 years. The prevalence of high-risk types of HPV DNA (HPV types 16 & 18) was 0.9%, compared to a group of women attending gynaecological outpatients in whom the prevalence of high-risk HPV DNA was 7%. The higher prevalence of HPV DNA in this group however, was strongly associated with a history of cervical pathology: 78% of women with HPV 16 and 18 had a history of CIN 1 – 3.

Reithmuller D et al[54] evaluated the use of HC II among 466 women attending their hospital for routine screening (mean age 36.2 years; range 16 – 76) and 130 with abnormal cytology referred to their colposcopy clinic (mean age 35.1; range 16 – 66). Using the 1x cut-off for a positive test, 17.8% of the women presenting for routine screening had a positive HC II test for high-risk types of HPV DNA. However, there was a statistically significant decrease in the prevalence of high-risk HPV DNA with age. For instance, in women aged 16 – 24 years, the prevalence of high-risk types of HPV DNA was 20.4% compared to 17.6% in women aged 35 – 44 years, 8.9% in the 45 – 54 age group and 5.8% in women older than 55 years ($p = 0.023$). Interestingly, the prevalence of high-risk HPV DNA in the 35 – 44 year age group was similar to the prevalence of HPV DNA in women aged 35 – 39 years old in the Khayelitsha study, but the prevalence in older women was very much lower.

There is little data on the prevalence of HPV DNA in healthy women in Africa. In Kenya, an HPV DNA prevalence of 20% (types 6, 11, 16, 18, 31 and 33 tested using PCR) was found among 77 women, attending a family planning clinic, with cytologically normal smears (mean age 25 years). On testing specifically for HPV 16, 18 and 33 using PCR, 10% were positive, which is considerably lower than found in our study, but the study sample is small and restricted to young women attending a family planning clinic and are unlikely to be generalisable to the population at large[55].

In a study conducted in Senegal[56] among 140 HIV positive commercial sex workers (CSWs), mean age 30 years, and 619 HIV negative CSWs, mean age 29 years, both high risk groups for sexually transmitted diseases, 56% of the HIV positive women and 40% of the HIV negative had positive HPV DNA tests (using PCR and Southern transfer hybridisation). However, the types of HPV DNA detected are not described in the article, and it is possible that these were low-risk types and they were a highly selected population at risk for sexually transmitted diseases.

In Cape Town, in a study of 262 women presenting for opportunistic screening and who had cytologically normal smears, the overall incidence of HPV DNA positivity was 13% using Southern blot hybridisation and 9% were positive for high-risk types of HPV[57]. Among women aged 20 – 39 years, 16% were positive for HPV DNA compared to 6% of women over the age of 39 years[57]. Southern blot hybridisation however is known to underestimate prevalence and the authors commented that the true prevalence of HPV in normal cervical smears in Cape Town women was probably much higher than reported in the study.

In a systematic review of HPV DNA testing in cervical screening programmes by Cuzick et al[58] studies of HPV DNA prevalence in 'normal' populations of women were reviewed. Only studies that used methods with some form of amplification of HPV DNA, either target amplification (as with PCR) or signal amplification (as with Hybrid Capture™), were incorporated in the review. In almost all studies prevalence of HPV DNA in 'normal' women decreased with age. In large studies that looked specifically at high-risk types of HPV, the prevalence was typically 10 – 30% at 20 – 30 years of age, falling to 3 – 10% over the age of 30 years. The authors comment that there is still controversy as to whether positivity falls still further over age 40 years or begins to rise again, and they indicate that more data is required for a fuller understanding of HPV prevalence in women over 40.

They further point out that other than disease status, the prevalence of HPV in a population is strongly influenced by lifestyle factors, particularly because it is well known that HPV is a sexually transmitted disease. The dominant epidemiological factors associated with high prevalence of HPV are the number of sexual partners in the last few years, and age of first intercourse.

The high prevalence of HPV DNA detected in our population of women may be explained by the relatively high proportion of women who began sexual intercourse as teenagers, as well as the high parity of the women and the high rate of other sexually transmitted diseases found in this population. The contribution of the 'male factor' was not evaluated in this study, but it is possible that male partners have multiple partners and act as a reservoir of infection of their female partners. Co-morbidity with other illnesses, such as Tuberculosis, which has an estimated incidence of over 500/100 000 of the population in Khayelitsha, may contribute to low immunity and inability to clear infection.

A further consideration is the impact of the HIV epidemic on the prevalence of detectable HPV in the population. A number of studies have shown increased detection of HPV DNA in HIV positive women

compared to HIV negative women, as well as significantly increased HPV detection in women with symptomatic HIV infection[59-63]. HIV testing was not performed on the women reported in this study, but in a subsequent study (not reported in this thesis), which was conducted from January 1998 to November 1999, 8% of women in the same age group and from the same population were HIV positive. There are no data on the prevalence of HIV in older women in Khayelitsha, however, data from anonymous testing of women attending antenatal clinics between January 1996 and end 1997, indicated an HIV prevalence of approximately 10%.

### 4.6.7 Issues to be considered in Assessing Cost-effectiveness of HPV DNA Testing as a Primary Screening Test

A cost benefit-analysis of HPV DNA testing as a primary screening test using the data presented here has not yet been performed. It is of value however to consider the issues that would be involved. Very few cost-analyses of HPV DNA testing have been performed, and most studies concerned with cost, base their conclusions on assumptions about the impact of HPV testing within existing cervical cytology programmes. An economic assessment of the introduction of HPV testing into a cervical cancer programme would require data on: 1] the costs of collecting specimens and the laboratory tests, that is, of the screening process itself, 2] the costs of following up and treating HPV positive women and 3] estimates of the effectiveness of HPV testing in reducing morbidity and mortality from cervical cancer.

A review of the costs of the cervical cytology programme of the National Health Service in the United Kingdom by Havelock[64] in 1994, found that the major cost is of screening itself, that is, taking and processing the smear. The cost of inviting the women for testing and making a diagnosis using colposcopy and histology was relatively small by comparison. It is likely that this will be true of HPV testing as well, although the costs of recalling women in developing countries has never been evaluated. Where modern communications such as telephones, faxes, email and postal services do not exist, recall of women is likely to require a more labour-intensive approach and therefore be less efficient.

The most important measure of the effectiveness of HPV testing will depend on the extent to which HPV testing will contribute to the reduction in the incidence of and mortality from cervical cancer. The more effective the screening activity, the more disease is diagnosed and treated. When this results in prevention of invasive cervical cancer, the cost of its treatment including hospitalisation, surgery and/or radiotherapy, will be saved. The more efficient the screening activity, the fewer women will be treated unnecessarily for disease that would not progress or would even regress in the absence of treatment. Ultimately the balance between the rate of identifying and successfully treating preinvasive disease, and the appropriateness of the screening programme combined with the cost of the programme will determine its cost-effectiveness.

In the case of HPV testing it is important to keep in mind that only a minority of women who test positive for HPV are likely to develop clinically significant disease, so many 'well' women will be labelled as having an abnormality. In addition, there is the problem that some stigma may be attached to testing positive for HPV since the virus is known to be sexually transmitted, and this may deter compliance with the screening programme. There are no studies on how women would react to the information that they are infected with a virus thought to be associated with a potentially life-threatening disease, and how this compares to the reaction to knowledge that they have an abnormal Pap smear.

There have been virtually no studies on the cost-benefit of HPV DNA testing, however Jenkins et al[65] used a stochastic model to predict resource use for HPV testing, comparing three alternative screening policies. They express the results in terms of the numbers of diagnostic tests needed and the impact on mortality from and incidence of cervical cancer, but do not explicitly estimate costs. The screening strategy of interest to this study, is one of routine screening by HPV testing with cytological follow-up for HPV positive women (similar to our Two-Stage Screening model). This screening strategy was estimated to reduce by about 25% the numbers of Pap smears required. The authors concluded that the cost-effectiveness of HPV testing as a routine screening test will depend substantially on the proportion of HPV negative cancers, as this would allow much longer screening intervals of up to 10 years. HPV testing is likely to be cost-effective (lower cost and improved quality control and automation) in comparison with cytology, where HPV negative precancers are less than 5%. The model however made no attempt to cost resource-use directly or to include treatment costs. The model is insufficiently detailed to derive estimates of cost per life-year gained or even of increased cancer detection.

Cuzick et al[58] in their systematic review, constructed two models that produced contrasting estimates of the cost-effectiveness of HPV screening. In model A they chose the most favourable combination of parameters i.e. progressive HPV infections have a long duration, the sensitivity of the HPV test is high, and the HPV prevalence in women without cervical neoplasia is relatively low. In model B, the opposite assumptions were made. For primary screening they considered 1] adding HPV testing to cytology and 2] replacing cytology with HPV testing. They considered 3 and 5 year screening intervals.

For both models, more intensive Pap smear screening (every 3 instead of 5 yearly screening) would prevent more deaths, 8.3/1000 women compared to 7.0/1000 (in a situation where the lifetime risk of dying from cervical cancer without screening is 10.9/1000 women), but this was less cost-effective. This is caused by a considerable decrease in screening and surveillance costs if women are screened less often, which outweighs the decrease in effects and the higher costs for invasive and advanced cancer.

According to model A, which was favourable for HPV screening, the combination of cytology and HPV testing performed once every 5 years reduced mortality more than did 3-yearly Pap smears. The

costs are only slightly higher. For screening with HPV testing only, the effects of 5-yearly screening are lower than for 3-yearly Pap smears (70 versus 76%), but the costs are reduced by 75%, resulting in a substantially lower cost-effectiveness ratio (£100 per life-year gained compared with £390 pre life-year gained). The lower costs of the HPV-only screening programme were mainly the result of lower screening costs and less (over) treatment of women referred without cervical neoplasia.

For model B, which was unfavourable for HPV screening, the use of HPV testing to supplement or replace cytology-based screening programmes, resulted in worse cost-effectiveness rates than cytology screening. Combined screening yielded a higher mortality reduction, but this was not proportional to the increase in costs if an HPV test were added to the Pap smear. For screening with HPV alone, both the effects were lower and the costs were higher. The higher costs were mainly the result of increased surveillance costs and lower savings in diagnosis and treatment costs.

The authors conclude that the results of their modelling show that for plausible values of prevalence, screening sensitivities and progression, HPV testing may be effective and cost-effective. There are uses of HPV testing that would provide benefits at a lower cost than many existing health care programmes. However, the wide range of results that came from using high and low estimates for these parameters show that more work is needed to allow modelling using more robust estimates.

## 4.7 Cervicography™ as a Primary Screening Test

At the outset, it is important to realise that Cervicography™ was never devised as a primary screening test, rather, it was designed as an adjunct to cytology. The basic concept behind Cervicography™ is that a person (physician, nurse, technician) without any knowledge of colposcopy can, after very brief training, take a picture of the entire cervix, which can then be sent to an expert for evaluation. In addition, it provides a permanent visual record of the cervix after the application of acetic acid[66]. The entire cervix is visible for evaluation and magnification is achieved by projecting the slide image onto a large screen and observing the projected picture from a short distance, giving ultimately a magnification of 16 x.

While we aimed to analyse the performance of Cervicography™ as a primary screening test in our study, an important additional reasons for including Cervicography™ was as a safety net to detect cancers that may have been missed by the nursing sister and as a means of quality control of DVI. From the outset, it was clear that the requirements of Cervicography™ suffer from the same drawbacks as cytology. The result is delayed and like cytology, the test requires subjective evaluation by experts. For low-resource settings without on-site trained Cervigram™ evaluators, this would require sending the film to other centres, or even other countries, for evaluation, greatly complicating the screening process.

Further, cervigrams are prone to a number of technical mishaps. We found that 333 (11.3%) of Cervigrams™ were unevaluable, compared to 22 (0.7%) unsatisfactory Paps and one sample unsuitable for HPV DNA testing. Of the unevaluable Cervigrams™, 60% were due to technical problems with the camera or with the electricity supply and the rest were due to poor quality Cervigrams™ caused by the presence of blood, mucus or the speculum obscuring the cervix. In a low-resource setting, this number of unsatisfactory evaluations would be unacceptably costly and would result in many women having to be re-screened, a particular problem for women who have to travel long distances to local clinics.

The performance characteristics of Cervicography™ have already been discussed in relation to DVI and HPV DNA testing and will not be repeated here. Suffice to say that we do not recommend Cervicography™ as a primary screening test in low-resource settings, but do recommend its inclusion in research studies incorporating DVI, as it provides the most accessible form of quality control of DVI testing.

## 4.8 Outcome of Treatment

### 4.8.1 Negative histology after LEEP

Overall 178 women were treated with LEEP in the study, but only 149 were treated on a 'see and treat' basis i.e. without histological sampling prior to treatment. Of the LEEPs performed without prior histological sampling, 41% had no histological evidence of either CIN or cancer, so-called, negative LEEPs. These women could be regarded as having been over-treated, despite having undergone colposcopic evaluation.

Negative histology after LEEP has been reported in a number of studies using a 'see and treat' approach. Murdoch et al[67] reported an overall 41% negative LEEP rate, in a highly selected group of women attending a colposcopy clinic because of abnormal cytology. In women who had had prior histological sampling, negative LEEP histology was found in 43% compared to 38% of those women treated with LEEP on a 'see and treat' basis. Of the latter women, 93% had decision cytology of *less than* CIN 1, 53% of CIN 1, 43% of CIN 2 and 18% of CIN 3. In view of these data, the authors cautioned against the 'see and treat' approach in women with low-grade referral cytology.

In a retrospective analysis of LEEP performed at the colposcopy clinic at Groote Schuur Hospital (GSH)[68], 18% of LEEPs performed after prior histological sampling (21 out of 116) were histologically negative compared to 14% who were treated on a 'see and treat' basis (16 out of 114). Of note, women are referred to the GSH colposcopy clinic with persistent LSIL (2 to 3 LSIL Paps over 12 to 18 months) or one Pap of HSIL or suspicious of malignancy. An additional finding in the study by Denny et al was that 25% of punch biopsies were falsely negative, which emphasises the point made by the authors, that a punch biopsy is only as reliable as the colposcopist's ability to identify the most abnormal area for biopsy.

A wide range of negative LEEP rates have been reported in the literature, from 5 – 41%. All of these studies have been performed in colposcopy clinics where women have been referred with abnormal cytology[69-74]. False positive cytology or colposcopy, false negative histology in the LEEP specimen and possible complete excision or spontaneous resolution of the lesion after prior biopsy are possible explanations for false negative LEEP histology. In addition, some series have reported negative LEEP histology where there has been extensive thermocoagulation preventing a histological diagnosis. In one series, incomplete sectioning of the LEEP specimen was responsible for negative LEEP in 2% of cases[75].

### 4.8.2 The Role of Colposcopy in Determining Histological Outcome

In our study, colposcopy was performed on women who were positive on any of the four screening tests. However, all colposcopies were blinded to the cytology result, unless a positive smear was the

only reason for referral for colposcopy. Colposcopy correctly identified all 12 cancers, one of these however, was diagnosed after a cone biopsy due to an unsatisfactory colposcopy. LSIL and HSIL were correctly identified in 27 and 26% of colposcopic assessments (where RCI was 3 or greater), respectively. However, colposcopy miss-classified 50% of the cases of histologically confirmed LSIL and 35% of the cases of HSIL as lacking cervical disease (RCI less than 3).

The outcome of the colposcopic assessment was strongly influenced by the nature of the positive screening test used for referral. For instance, among women with positive DVI examinations or Cervigram™ evaluations, just under 30% with a RCI ≥ 3 had HSIL confirmed histologically for both tests. In contrast, approximately 40% of women with positive Pap smears and positive HPV DNA tests had HSIL. These results may have influenced the colposcopic assessment in that the colposcopist had a greater expectation of finding disease with the latter two tests and may have interpreted aceto-white lesions differently.

Colposcopy is optimally performed in the absence of infection and under ideal hormonal conditions, for instance, during day 8 and 12 of the menstrual cycle, when the cervical mucus is most translucent and abundant and the external os is most widely dilated. These conditions were not met in the context of our study. Although colposcopy was deferred in some women for treatment with antibiotics or local estrogen cream, the majority of colposcopies were performed when women presented without deferring assessment, regardless of the presence of atrophy or inflammation. This policy was followed in order to minimise defaulting and to truly evaluate the provision of an on-site screening and treatment service in a low-resource setting. Performing colposcopy under these circumstances may explain the high rate of negative histology after LEEP, or, by inference, the high false positive rate of colposcopy.

In a meta-analysis of colposcopy for the diagnosis of SIL by Mitchell et al[76], 9 out of 81 studies were selected for incorporation into the analysis as they distinguished histologically normal cervices from all other diagnoses. The individual estimations of sensitivity of diagnostic colposcopy were high (range 87 - 99%), whereas those of specificity were lower and wider (range 23 - 87%). Similarly, among 8 studies with fully separated disease categories, for distinguishing normal cervix, atypia and LSIL from HSIL and cancer, the estimations of sensitivity of diagnostic colposcopy were high (range 64 - 99%), whereas those of specificity were lower (range 30 - 93%). High-grade lesions appeared to have distinguishing characteristics that allowed them to be better separated from LSIL than was possible for separating LSIL from a normal cervix.

In an overview of the literature from 1972 to 1995 on the positive predictive value of colposcopic examination[77] colposcopy correctly identified CIN 3 in an average of 78% (range 44 – 93%) of cases examined among selected women in colposcopy clinics. The corresponding figures for CIN 2 and 3 were 43 and 59% respectively. In other words, the PPV of colposcopy was highest in women with high-grade disease.

Colposcopy in the Khayelitsha study was performed in women selected on the basis of four different screening tests with varying sensitivities and specificities. In the majority of cases the colposcopist was blinded to the result of the Pap smear. However, even with the use of colposcopy, significant numbers of women were over-treated or miss-classified by the colposcopic diagnosis.

### 4.8.3 Complications of LEEP

Of the 178 women who underwent LEEP, excessive bleeding at the time of the procedure was recorded in just under 5% of the women (although none of these women required admission to hospital and the bleeding was controlled at the time of the procedure). A further 49% of women had moderate bleeding that was controlled with ease at the time of the procedure (estimated at less than 100 mls of blood) and in 46% of women there was no bleeding at all. While cramping and pain related to the procedure was recorded at the time of LEEP in 28% of the women, none of the women required medication to control pain and the pain was not persistent. No serious complications at the time of LEEP were recorded.

A limitation of our data on post-LEEP complications is that these were recorded by interviewing the women at 4 months. All women were strongly advised to return to the project if any complications developed and the author's emergency telephone number was given to the patients should complications occur. While women did return to the project when complications occurred, this was not recorded in a systematic manner. Hence, our data on post-LEEP complications are limited by patient recall and by the fact that we only have information on women who returned for post-LEEP follow up. It is possible that the complication rate was higher among those who did not return for follow up, however this is speculative.

Of the 141 women who returned for the post LEEP visit at four months, 17% (n = 24) reported that they required medical treatment after LEEP. Of these women, 14 developed infection (just under 10% of the women treated with LEEP who were followed up). 7 had excessive bleeding and 3 women had unspecified complaints. Twenty-three of the 24 women attended primary care facilities for treatment of complications and one woman with excessive bleeding was referred to the emergency unit at Groote Schuur Hospital.

Cervical stenosis was recorded in 5.7% (8/141) of the women who returned for their 4 month post-LEEP check-up. A further 4 (6.3%) cases of cervical stenosis were diagnosed in the 64 women who returned for their 10 month post LEEP check-up.

In a randomised trial of LEEP, Cryotherapy and Laser vaporisation of the cervix, Mitchel et al[78] reported complications from LEEP in 7.6% (10/130) of women treated. The majority of the complications related to bleeding (70%), there was one case of infection, one woman complained of severe pain and required treatment and one woman subsequently developed cervical stenosis.

Ferenczy et al[75] reported on 1070 women who underwent LEEP and who returned for post-LEEP follow up. Complications were recorded in 7% (n = 71) of women; 37 had significant intra-or post-operative bleeding, and one woman required admission to hospital to control bleeding. Seven women developed a purulent vaginal discharge and pelvic pain within one week of treatment. A further 13 (1.2%) women developed cervical stenosis and they noted that 11 of these women were over the age of 45 and were not on hormone replacement therapy.

The complication rate in the Khayelitsha study is higher than reported in series conducted in colposcopy clinics in developed countries, particularly of cervical stenosis. Considering the relatively high rate of lower genital tract infection in women in our study, this is perhaps not surprising. However, there were no serious complications noted at the time of the procedure and only one woman, that we know of, required admission to hospital for management of excessive bleeding.

### 4.8.4 Persistence/Recurrence of Disease Post-LEEP

Overall, SIL was diagnosed in 9 (6.4%) of the 141 women who returned for their 4-month post-LEEP check-up. Only one woman had HSIL confirmed histologically and this woman had HSIL diagnosed in the original LEEP specimen. The other 8 women had LSIL confirmed histologically at the 4-month visit, and in two cases the original LEEP diagnosis was negative. At the 10-month check-up, one case of HSIL was diagnosed in a woman whose original LEEP histology was negative (1.2% of women who returned for follow-up). LSIL was diagnosed in a further three women (4.6% of the women who were followed-up), 2 with original LEEP histology of HSIL and one with LSIL.

These rates of persistence/recurrence compare favourably with series published from colposcopy clinics in developed countries. Ferenczy et al[75] reported a 'cure rate' of 93% of women with LSIL treated with one LEEP procedure (7% persisted) and 90% of women with HSIL treated with one LEEP procedure. The figures increased to 96 and 93% respectively when repeat treatments were performed.

Flannelly et al[79] reported on a retrospective analysis of the first 1000 women treated in their unit with LEEP from 1989 – 1991. Follow-up cytology was available for 977 women, 975 who had at least one follow up smear, 930 had at least two, 845 had three and 686 had more than three follow up smears. Overall 8.2% of follow up smears were found to have CIN. Of women with initial pathology of CIN 3 in the LEEP specimen, 8% persisted compared to 4% of women with initial histology of CIN 1.

Hulman et al[80] reported on a retrospective review of 669 women treated with LEEP and followed up for 1.5 to 3.5 years. Persistence or recurrence of histologically confirmed CIN was 6.7% for initial pathology of CIN 1, 13.4% with CIN 2 and 21.7% for CIN 3.

Our patients were followed up for a 10 month period post-treatment and this is probably too short a time period to assess long-term success or failure of LEEP. Flannelly et al reported that the majority of

cytological abnormalities post-LEEP occurred within 18 months of treatment, thereafter the rate decreased but remained steady for a further 48 months. Longer term follow up from their study is still awaited.

In summary, LEEP was found to be a safe procedure in our hands with a cure rate of over 90% for 10 months of follow-up. Unfortunately, significant numbers of women did not return for both the 4 and 10 month check-ups, which emphasises the importance of adequate initial treatment as many women default follow-up.

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# CHAPTER 5

## 5.1 Conclusions

The main conclusions from the study are the following:

1. Locating a screening service close to where women live with a rapid turn around of results (2 – 6 days in this study) resulted in an excellent follow up rate of just over 90% of screen positive women. The longer the delay between women being screened and treated the higher the default rate. The majority of the defaulters were the poorest and most marginalised women in the community, indicating that this group of women require special attention. The relatively poor follow up rates post-treatment have important implications for mass screening programmes in resource-poor settings, where recurrence/persistence rates of disease of at least 5 – 10%, if not higher, would be expected.

2. Using a mid-level clinician, such as a nursing sister, to screen women proved to be feasible. In addition, Community Health Workers were found to be a valuable resource in the project and were eminently trainable as both educators and administrators. The sustainability however of a vertical service in low-resource health care settings, as was delivered in this study, is questionable.

3. DVI, HPV DNA testing at the lower cut-off level (1x) and cytology had equivalent sensitivities for the detection of HSIL and cancer (range 67 – 78%). However, both DVI and HPV DNA testing (1x) had significantly lower specificities and PPVs than cytology. If a screening strategy were to be based on a 'Test and Treat' protocol using DVI or HPV DNA (1x) as the primary screening tests, not only would a relatively high percentage of the screened population be referred for treatment, but also large numbers of women without cervical disease would undergo treatment. While DVI and HPV DNA testing may identify two-thirds or more of the high-grade lesions in the screened population, only 11% and 14% of the screen positive women would have high-grade disease, using DVI and HPV DNA testing (1x) respectively. Increasing the threshold for a positive HPV DNA test to 10 x the positive control (that is, to high levels of HPV DNA), significantly reduces sensitivity (to 50%) with a concomitant increase in specificity and PPV. This approach would reduce both the number of women referred for treatment and the number of women over-treated, but half the cases of disease would be missed.

4. Using HC II, the sensitivity of HPV DNA testing is superior to that of expert cytology but the specificity is lower. The specificity of HPV DNA testing however can be altered by altering the cut-off level to define a positive test.

5. Cervicography™ is unlikely to have utility as a primary screening test, because of its relatively poor test characteristics, the need for experts to read the slides, the technology required and the fact that the result is delayed. To enable a quicker turnaround of results, large numbers of local

'experts' would need to be trained. Cervicography™ does however have a role as a possible method of quality control of DVI and the slides can be used to train nursing sisters in DVI in the context of a research study

6. Different screening strategies need to be evaluated according to existing health resources. Two-stage screening offers many advantages, particularly in very low resource settings, where facilities for laboratory-based tests are limited. Two-Stage Screening using any of the six sequences, dramatically reduces the number of women referred for treatment and, if DVI is used as the initial screen, reduces the number of more expensive tests e.g. Paps or HPV DNA tests required. The loss of sensitivity with Two-Stage Screening may be acceptable in settings where cytology services are limited or do not exist at all. The higher specificity of Two-Stage Screening however, will prevent large numbers of women undergoing unnecessary treatment.

7. On-site provision of colposcopy and LEEP used in a 'see and treat' context was safe and effective in our hands. The main concern was the relatively high percentage of cervical stenosis in treated women, which may lead to inadequate follow up or gynaecological complications such as haematometria, in these women. LEEP however, may not be the ideal method of treating preinvasive disease in low resource settings, particularly as it requires the use of local anaesthetic and a fair amount of technical skill. Alternative ablative therapies, such as cryotherapy that do not require local anaesthetic and do not rely on electricity may be more appropriate.

## 5.2 Recommendations

This study has shown the potential utility of both DVI and HPV as alternatives to cytology in resource-poor settings. In order to extrapolate these data into day to day clinical practice, further studies on the performance of the screening tests in the field (i.e. without the close supervision of a well-controlled research study) need to be conducted. Further, it is essential that standardised methods of interpreting aceto-whitening are developed, as well as curricula for training of health workers to perform DVI.

While both tests will identify most cases of high-grade disease in the screened population, a significant number of women would be over-treated if treated simply on the basis of the screening tests. Considering the burden of cervical cancer, would this necessarily be a negative outcome?

It is with this background that we recommend further studies designed to answer the following questions be initiated. The specific questions that need to be addressed are the following:

1. What would be the safety, efficacy and acceptability (to women and to health care workers) of a 'Test and Treat' protocol in which women would be treated by nursing sisters, on-site, on the basis of a positive screening test alone (eg DVI or HPV)? Would such a protocol reduce the incidence of and mortality from cervical cancer? Would women agree to be tested on a large-scale for a known sexually transmitted disease such as HPV? How would this impact on compliance with the

programme? Finally and, ultimately of greatest concern to health policy planners, what would be the costs per life saved of a 'Test and Treat' protocol, compared to either no screening programme or a cytologically-based programme?

2. What would be the complication rate of treatment when performed in a low-resource setting by nursing sisters without colposcopic guidance? Complications that will need to be evaluated include pelvic inflammatory disease, cervical stenosis, and bleeding requiring additional medical or surgical management.
3. Could either DVI or HPV DNA testing be used effectively for post-treatment follow-up of women?

Further, efforts need to be directed towards developing an even simpler method of HPV DNA testing than is currently available, specifically a side-room type of test that can give an immediate result or a fully automated process for testing. In some low resource settings, such as in the emerging economies, it may be possible to establish HPV laboratories although, current costs of the kits and the capital outlay may prove to be prohibitively expensive. In very low resource settings, such as are found in Africa, setting up HPV laboratories even with lower level technicians is unlikely to be feasible in the next few decades.

Of critical importance though, is whether there is a significant cost-benefit in terms of lives saved using these alternative methods compared to cytology. The acceptability of these tests to the screened population, as well as test and treat protocols need to be rigorously evaluated.

Finally, an important component of the success of this project, was that from the outset, the women and the community were consulted. Our approach was to encourage participation of the women being screened and we viewed them as our 'teachers'. In other words, it was the women themselves who showed us 'the way' in terms of developing educational material, adjusting the service to suit their needs and co-operating with non-western health care providers in the area. For screening to be successful, it is essential to consult with the women and their community and to design a screening programme which is not only acceptable to women, but recognises and responds to the many barriers that prevent, particularly poor women, from joining or complying with a screening programme.

## 5.3 Final Comment

In an era during which prophylactic HPV vaccines are being actively developed, it is tempting to halt the search for alternative methods of screening to prevent cervical cancer. It is important however to remember that the clinical utility of HPV vaccines is possibly 10 to 20 years away. In addition, the infrastructure to distribute HPV vaccines will need to be developed, and some have estimated this will take at least another 60 years. In the meantime, we have a responsibility to continue the search to develop methods of screening for women living in the poorest countries of the world, that are effective, safe and acceptable to the women being screened.

# Appendix A
## *Enrolment Questionnaire (Form 01)*

| 0 | 1 |
|---|---|

Patient PATNO Name:_____

Day Month Year

Date of interview

Interviewer Name: _____ .....13

1. Year of birth: _____ 14

How old are you? (age in years) _____ 16

2. Were you born in Cape Town?

Yes ..... 1
No ..... 2

..... 18

IF NO, how long have you been in Cape Town?

0-1 year..... 1
1 year to 5 years ..... 2
More than 5 years..... 3

..... 19

3. What is the highest level of schooling you have completed?

No school ..... 88
std 1 or less ..... 01
std 2 ..... 02
std 3 ..... 03
std 4 ..... 04
std 5 ..... 05
std 6 ..... 06
std 7 ..... 07
std 8 ..... 08
std 9 ..... 09
std 10 ..... 10
Any tertiary/university ..... 11 20

4. Are you married?

Yes ..... 1
No .....
2..... 22

IF YES, how much of the year does your husband live with you?
All year round (9 or more months per year) ..... 1
Greater than 1 month but less than 9 months ..... 2
One month or less ..... 3
..... 23

IF NO, which best describes your situation?
Live with boyfriend ..... 1
Separated/ Divorced (not remarried)..... 2
Widowed (not remarried) ..... 3
Single ..... 4
Other _____ 5
..... 24

5. Do you have a job?
Yes ..... 1
No .....
2..... 25

IF YES, what is your occupation

_____

6. How do you get most of your money?
Own job ..... 1
Selling or making things from home (Informal sector) ..... 2

Husband or boyfriend's job ..... 3
Help from family or friends ..... 4
Disability, pensions, unemployment etc ..... 5
Other _____ 6
..... 28

7. Where are you living now?
House ..... 1
Shack on a serviced site ..... 2
Shack on an unserviced site/
or other open land ..... 3
Other_____ 4
..... 29

8. At your living quarters, do you have running water?
Yes, indoors ..... 1
Yes, on property but not indoors ..... 2
No ..... 3
..... 30

9. At your living quarters, do you have electricity?

Yes ..... 1
No ..... 2

..... 31

10. How old were you when you became pregnant for the first time? ..... 32
(Write 00 in blocks if never pregnant.)

IF EVER PREGNANT

10a. How many times have you been pregnant? ..... 34
(Write 00 in blocks if never pregnant)

IF EVER PREGNANT

10b. How many times have you had a live birth? ..... 36
(Write 00 in blocks if no live birth)

11. How old were you when you first had sexual intercourse? ..... 38
(This includes sexual intercourse with a man, even if you were an unwilling partner.)

12. How many different men have you had sex with in the last month? ..... 40
(Write 00 in blocks if no sex in the last month)

13. How many different men have you had sex with in your entire life? ..... 43

14. Have you drunk any alcohol in the last month?

Yes, moderate or large amount (4+ drinks on a single occasion)..... 1
Yes, small amount (0-3 drinks on a single occasion) ..... 2
None at all ..... 3 ..

..... 46

. Have you **EVER** used any method of birth control?

Yes ..... 1
No ..... 2 (IF NO, SKIP TO Q 18)

..... 47

15a. IF YES, have you EVER used the following?

Birth control pills Yes ..... 1 No ..... 2

..... 48

Injection (Depo) Yes ..... 1 No ..... 2 ..... 49

Condom Yes ..... 1 No ..... 2 ..... 50

Sterilization Yes ..... 1 No ..... 2 ..... 51

Other _____ Yes ..... 1 No ..... 2 ..... 52

15b. IF YES TO ANY ABOVE, have you used the following IN THE LAST 3 MONTHS?

Birth control pills Yes ..... 1 No ..... 2 ..... 53

Injection (Depo) Yes ..... 1 No ..... 2 ..... 54

Condom Yes ..... 1 No ..... 2 ..... 55

Other _____ Yes ..... 1 No ..... 2 ..... 56

16. Do you smoke regularly? (Regularly means at least daily on average and smoking includes cigarettes, pipe, rolled cigarettes)

Yes ..... 1
No ..... 2
..... 57

17. For what reason did you come to the clinic today?

Pap smear (Cervical cancer) Screening ..... 1
Gynecological complaint ..... 2
Other medical condition ..... 3
Other _____ 4
..... 58

# Appendix B
## *Clinical Examination: Colposcopy (Form 05)*

| 0 | 5 |
|---|---|

Patient Name:_____ PATNO 3 | | | | |

Day Month Year

Date of clinical exam 7 | | | | | | |

Clinician:_____ 13

1. Are the results of the pap smear available (when this exam is done)?
   - Yes ..... 1
   - No ..... 2 ..... 14

2. Colposcopic exam:
   - Satisfactory ..... 1
   - Unsatisfactory ..... 2 .....15

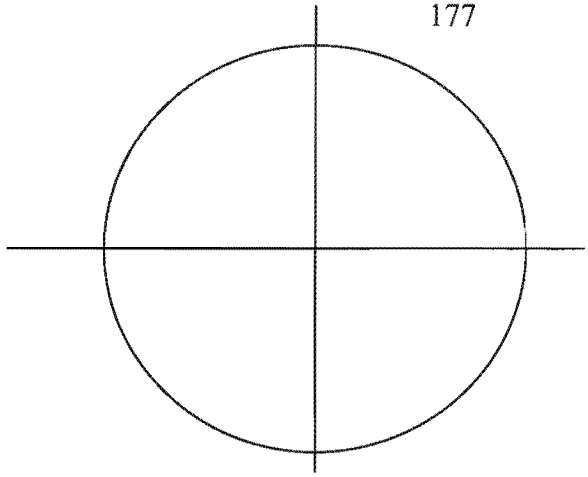
3. Are there cervical lesion(s)?
   - Yes ..... 1
   - No ..... 2 .....16

3a. IF YES, score the most severe lesion using the following criteria:

| | | | |
|---|---|---|---|
| Margin | **0** Condylomatous / micropapillary<br>Indistinct borders<br>Jagged, angular lesions | **1** Regular lesions with<br>th outlines | **2** Rolled, peeling edges |
| Color | **0** Shiny snow white<br>Indistinct acetowhitening<br>Semi-transparent | **1** Shiny, off-white<br>Intermediate white | **2** Dull, oyster grey |
| Vessels | **0** Uniform, fine caliber punctation or<br>Randomly arranged with poorly formed patterns. Non-dilated capillary loops arranged<br>defined patterns | **1** Absence of surface vessels | **2** Definite mosaicism. Individual vessels dilated, in well |
| Iodine staining of Staining grade | **0** Positive iodine uptake (mahogany brown)<br>Negative iodine uptake in low grade lesion (<2/8) yellow | **1** Partial iodine uptake<br>Variegated, tortoise shell appearance | **2** Negative lesion high (>3/8)<br>Mustard |

Total Reid

b. IF YES, diagram of lesion(s):



4. IF REID ≥ 3, was LLETZ performed?

Yes ..... 1
No ..... 2 ☐
..... 22

4a. IF NO, select reason why not:

Patient refused local anesthetic ..... 1
Other infections ..... 2
Patient unsuitable ..... 3
Other _____ 4 ☐
..... 23

4b. IF YES, did patient complain of:

Pain /Cramps Yes .... 1 No .... 2 ☐
..... 24

Light-headedness or
(feeling faint) Yes .... 1 No .... 2 ☐
..... 25

Other _____

4c. IF YES, was there bleeding?

Yes, ≥ 100 ml ..... 1
Yes, < 100ml ..... 2
No ..... 3 ☐
..... 26

5. IF REID ≤ 2, was punch biopsy done?

Yes ..... 1
No ..... 2 ☐
..... 27